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CONTENTS

Streptokinase-Streptodornase in the Local Treatment of Suppurative Extrapulmonary Tuberculosis. George N. Hazlehurst 1
Studies on the Intrathecal Use of Streptokinase-Streptodornase in Tuberculous and Other Types of Bacterial Meningitis. George N. Hazlehurst 12
The Surgical Treatment of Spontaneous Idiopathic Hemopneumothorax. Wesley Fry, William L. Rogers, Gerald L. Crenshaw, and Harry C. Barton 30
The Relationship of Nonspecific and Specific Factors in the Tuberculin Reaction. A Review. J. Pepys 49
The Cultural Characteristics and Animal Pathogenicity of an Atypical Acid-Fast Organism Which Causes Human Disease. Ann Pollak and Victor B. Buhler 74
Observations on a Pleomorphic Mycobacterium Isolated from Mouse Brains. I. Cultural and Morphologic Characteristics. W. A. Vischer, J. Daniels, and A. M. Pappenheimer 88
Observations on a Pleomorphic Mycobacterium Isolated from Mouse Brains. II. Pathology and Comparison of Lesions with Those Produced by Certain Other Strains of Mycobacteria. A. M. Pappenheimer, J. Daniels, and W. A. Vischer 97
Effects of Sunlamp Irradiation on the Viability, Virulence, and Biochemical Activity of *Mycobacterium Tuberculosis*. William Segal and Hubert Bloch 112
Case Reports:
Isolation of *Allescheria Boydii* from Sputum. Joseph Creitz and H. William Harris 126
Recurrence of Coccidioidal Cavity Following Resectional Surgery. Leroy Hyde 131
Pulmonary Resection in Silicotuberculosis. Jesse P. Chapman, Jr., and John R. Bumgarner 137
Editorial: Can We Accelerate Tuberculosis Research? Floyd M. Feldmann 140
Notes:
Experience with the Use of Quartz Dust in an Attempt to Test the Viability of Tubercle Bacilli in Resected Closed Tuberculous Lesions Following Long-Term Antimicrobial Therapy. John D. Steele and Dana B. Claudon 144
Books 146
American Trudeau Society:
Organization and Committee Structure, 1954–1955 148
Notices 160
Abstracts 1

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*Heck, W.E.; Lynch, W.J., and Graves, H.L.: Acta oto-laryng. 41:416, 1953.

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ERRATUM

In Volume 70, No. 3, 1954, "The Chemotherapy of Pulmonary Tuberculosis with Pyrazinamide Used Alone and in Combination with Streptomycin, Para-Aminosalicylic Acid, or Isoniazid" by William S. Schwartz and Ralph E. Moyer, the dosages in the first sentence on page 420 should read 42.4 *mg.* and 44.8 *mg.* instead of 42.4 gm. and 44.8 gm.

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STREPTOKINASE-STREPTODORNASE IN THE LOCAL TREATMENT OF SUPPURATIVE EXTRAPULMONARY TUBERCULOSIS[1, 2]

GEORGE N. HAZLEHURST

(Received for publication August 24, 1954)

INTRODUCTION

This report deals with the use of streptokinase-streptodornase[3] in the local treatment of suppurative tuberculous lesions. The majority of the case material to be presented consists of patients with suppurative tuberculous adenitis (seventeen nodes in 11 patients). In addition, there are included: one patient with Pott's disease and psoas abscess, one patient with an axillary cold abscess secondary to tuberculosis of the shoulder, and one patient with tuberculous tenosynovitis.

A preliminary report dealing with the use of streptokinase-streptodornase in tuberculous adenitis has appeared elsewhere (1). Reference may be made to that article for photographs of three of the treated cases presented below (*Cases 4*, *6*, and *8*).

MODE OF APPLICATION

In order to find the most effective method of application of the enzymes to the diseased area, several different techniques were given a trial. Initially, the fluctuant nodes were aspirated with a needle traversing surrounding healthy tissue and were then irrigated with enzyme solutions. This method was unsatisfactory for two reasons: the needle tracts frequently became infected with the tuberculous process; and the interior of the node could not be visualized for the purpose of observing therapeutic progress. Accordingly, a technique of widely incising the fluctuant area was adopted, and enzyme treatment was accomplished by local irrigation, using small plastic catheters. This technique provided adequate drainage and direct visualization of the local effect of the enzymes. Irriga-

[1] From the Department of Medicine of the New York University College of Medicine, and the Third Medical Division of Bellevue Hospital, New York, New York.

[2] This study was supported by a contract with the Medical Research and Development Board of the Office of the Surgeon General, Department of the Army, U. S. A.

[3] Supplied by Lederle Laboratories under the trade name "Varidase."

tions were carried out almost daily and were usually prolonged over a period of fifteen minutes to wash out any material which might be liquefied by the enzymes. The irrigation solution used consisted of 30 to 60 ml. of saline containing one or two ampules of streptokinase-streptodornase. (Each ampule contained from 150,000 to 300,000 units of streptokinase and from 75,000 to 250,000 units of streptodornase, depending upon the batch used.) When all necrotic and caseous material disappeared from the base of the node and was replaced by healthy-looking granulation tissue, enzyme irrigations were stopped and the area was left to heal by itself.

Later it was found that the enzymes could be successfully incorporated in a water-soluble surgical lubricating jelly. The preparation selected was Lubafax®. After the addition of a small amount of Na_2HPO_4 as a buffer, the pH of the jelly was adjusted to pH 7.5. To 30 cc. of jelly thus prepared in a sterile glass container, one ampule of streptokinase-streptodornase dissolved in 0.5 ml. of saline was added and mixed thoroughly with a sterile glass rod. The enzymes in this preparation were surprisingly stable and retained their potency for at least three weeks under refrigeration.

Subsequently a more flexible jelly vehicle was adopted. It consisted of a mixture of gum tragacanth and sodium alginate in isotonic Sorensen's buffer, adjusted to pH 7.5. Tenaciousness or thickness of the jelly could be varied in degree by increasing the proportions of tragacanth or alginate, respectively, and the jelly vehicle could be autoclaved for sterilization.

By the use of the jelly preparations, the daily treatment of the incised node was greatly facilitated. A small amount of the jelly was placed within the open ulcer and the area covered with a bleb of paraffin paper to prevent absorption of the jelly into the overlying gauze dressing.

In the 3 cases representative of other types of extrapulmonary tuberculosis presented below, irrigation techniques were employed since the physical characteristics of the lesions did not lend themselves to effective jelly application. The mode of application in these 3 cases will be discussed as they are presented.

RESULTS

Suppurative Tuberculous Adenitis

The pertinent data on all patients who were treated are given in table 1. A total of seventeen nodes in 11 patients have received enzyme therapy. In all but one patient, the diagnosis was proved by the demonstration of tubercle bacilli by microscopy or culture. In *Case 6*, the demonstration of unequivocally characteristic changes in biopsy material was accepted as evidence for tuberculosis.

Streptomycin was given concomitantly with the enzyme therapy in the first 5 patients listed in the table. This was done as a precautionary measure to prevent possible spread of the disease from the local area during enzyme therapy. Later, however, when the lesions were incised and drainage appeared adequate, it was decided that streptomycin was no longer necessary for this purpose. Antituberculous drugs were not used in the next 4 patients (*Cases 6* through *9*), and no

gross differences in healing time were noted. In the last 2 cases in table 1, however, response to enzyme treatment was slow. Factors relating to management of these 2 cases are discussed below.

Additional facts on representative cases of this group seem worthy of brief comment.

In *Case 2*, healing following onset of enzyme therapy was hindered by the presence of *Staphylococcus aureus* in the wound. This was controlled by the administration of chlortetracycline (Aureomycin®) orally over a five-day period, after which healing progressed rapidly. This was the only case in this series of nodal lesions in which secondary infection played a role.

The right suboccipital node in *Case 4* had been allowed to break down spontaneously, resulting in a deep ulcerated lesion which exuded copious amounts of creamy brown discharge. Within five days after beginning enzyme irrigation, drainage stopped and the visible portions of the ulcer appeared clean. Three weeks later only a thin scar remained and there was no palpable induration.

The lesion in *Case 6* was one of the largest treated. It appeared to be an isolated lesion without adjacent induration or other palpable nodes. It was filled with sanguineopurulent fluid as well as numerous particles of necrotic and caseous material. The base of the node was filled with soft mushy granulomatous tissue lined at the center by caseous adherent matter which upon removal revealed a small sinus tract extending directly inward for a distance of 2 cm. from the base. After two weeks of treatment, the base of the node showed only healthy-looking exuberant granulation tissue, and the sinus tract could no longer be probed. Progressive healing took place, and after six weeks only a thin scar remained.

In *Case 8* multiple lesions were present. Two separate fluctuant nodes were successfully treated with enzyme jelly, and two small connecting draining sinuses closed after five irrigations with enzyme solution. This patient had been treated with streptomycin for several months prior to enzyme therapy without noticeable response of the local tuberculous infection.

In *Case 9*, the use of enzyme jelly was accompanied by transformation of a dirty gray granulomatous nodal base to exuberant red granulations within a week. Epithelization of the area progressed at such a slow rate, however, that the edges of the lesion were at length sutured together. The patient left this country immediately thereafter, and follow-up observation has not been possible.

In *Cases 10* and *11*, healing was considerably prolonged compared with the other treated nodes. This was believed to be due to the presence of a considerable amount of induration of the tissues surrounding the nodes. In *Case 10*, tubercle bacilli were cultured at widely spaced intervals during the prolonged course of treatment. In this case antituberculous drugs were purposely withheld in order to evaluate enzyme therapy. Although the lesion eventually healed under this treatment, it was believed that healing might have been hastened by the use of antimicrobials. In *Case 11*, the patient received several antituberculous drugs during the period of enzyme therapy. Healing in this case, however, was delayed by premature closing of the top of the lesion. When, by mechanical means, healing from the base of the lesion was permitted, complete closure of the lesion was rapid.

All of the nodes treated with streptokinase-streptodornase have remained healed during the follow-up periods specified in table 1. A few of the patients

TABLE 1

SUMMARIZED DATA ON 17 SUPPURATIVE TUBERCULOUS LYMPH NODES TREATED WITH STREPTOKINASE-STREPTODORNASE

Case Number	Age	Location	Duration	Size	Character	Mode of Treatment	Number of Treatments	Period of Treatment	Healing Time	Follow-up Period
1	8 months	Right anterior cervical	3 weeks	2 x 2 cm.	Marked fluctuation, thin reddened skin	Needle aspiration and irrigation	2	3 days	1½ weeks	11 months
2	4	Left preauricular	10 weeks	1 x 2 cm. 2 cm. deep	Completely incised open ulcer	Irrigation	9	12 days	3 weeks	3½ years
3A	1	Right submandibular	4 weeks	2 x 3 cm.	Marked fluctuation with thin, reddened skin overlying both nodes	Needle aspiration and irrigation; later, small incisions of both areas	9	25 days	5 weeks	4 years
3B		Left submandibular	4 weeks	2 x 3 cm.			6	10 days	3 weeks	4 years
4A	11	Right suboccipital	5 weeks	2 x 4 cm. 3 cm. deep	Open draining ulcer (spontaneous breakdown)	Irrigation	7	11 days	3 weeks	4 years
4B		Mid-suboccipital	4 weeks	3 x 3 cm.	Marked fluctuation deep in subcutaneous tissue	Needle aspiration, small incision, irrigation	2	10 days	3 weeks	4 years
5	6	Right anterior cervical	3½ months	2 x 2 cm.	Moderate subcutaneous fluctuation, reddened skin	Small incision and irrigation	3	9 days	3 weeks	4 years
6	2	Left anterior cervical	4 weeks	4 x 4 cm. 2 cm. deep	Large, protuberant, very fluctuant; tissue-thin skin	Incision and drainage, irrigation	17	25 days	6 weeks	1 year
7A	2	Right anterior frontal	6 weeks	1.5 x 1.5 cm.	Fluctuant, thin reddened skin, small sinus	Irrigation and jelly, later excision	4	10 days	2 weeks	5 months
7B		Right preauricular	5 weeks	2 x 3 cm.	Moderate fluctuation and reddening	Small incision and irrigation	7	10 days	2 weeks	5 months
7C		Right subauricular	5 weeks	3 x 6 cm.	Very fluctuant, reddened overlying skin	Small incision, irrigation and jelly	9	20 days	4 weeks	5 months

8A	8l	Right sub-auricular	6 weeks	3 x 4 cm. 3 cm. deep	Slightly fluctuant, small deep draining sinus	Incision and drainage, jelly	7	7 days	2 weeks	1 month
8B		Right supra-clavicular	2 weeks		Two small connected draining sinuses, 4 cm. apart	Irrigation	5	10 days	2 weeks	1 month
8C		Supra-sternal notch	1 week	2 x 4 cm.	Upper one-half indurated; lower one-half fluctuant	Incision and drainage, jelly, irrigation	6	12 days	3 weeks	2 weeks
9	23	Right supra-clavicular	6 months	2 x 6 cm.	Small draining sinus with excavation	Incision and drainage, jelly, irrigation	9	4 weeks	(*see* text)	none
10	10	Right posterior cervical	1 month	4 x 6 cm.	Fluctuant, surrounding induration, thin red skin	Incision and drainage, irrigation, jelly	daily	7 weeks	9 weeks	1½ years
11	9 months	Left sub-mandibular	2 weeks	3 x 4 cm.	Mass of fluctuant nodes matted together, tender	Incision and drainage, irrigation, jelly	daily	5 weeks	10 weeks	2 weeks

have developed tuberculous nodes in other locations, but none of the treated lesions has broken down.

As may be seen in the table, the healing time varied considerably from node to node. In general, the smaller the treated area, the faster healing took place. The amount of induration of tissue adjacent to the node appeared to be equally important. It should be emphasized that the enzyme action is purely local in scope, and areas of disease not in direct contact with the streptococcal concentrates remain unaffected. In a few cases, following incision and drainage of the fluctuant areas, adjacent or subjacent indurations tended to subside if free drainage was allowed to continue for a few days. In other cases, resolution of the surrounding induration was extremely slow.

In general, the fastest healing was seen in the isolated fluctuant node which had very little surrounding induration. In those cases, healing time varied with the size of the node. Slow healing was seen in those nodes with considerable degrees of adjacent induration, indicative of disease out of reach of the enzyme material. In those cases, the size of the lesion was of secondary importance in healing time.

Concomitant use of antituberculous drugs did not appear to be necessary in the isolated, nonindurated lesion. It was believed, however, that these drugs were indicated when evidence for active tuberculous disease was present deep in tissues adjacent to the node, either in the form of persistent induration or the presence of a feeding focus of tubercle bacilli as evidenced by persistently positive cultures.

Miscellaneous Suppurative Tuberculous Lesions

Case 12: Axillary cold abscess. This 56-year-old white male had developed tuberculosis of the left shoulder joint four years previously. Treatment had consisted of surgical resection of the head of the humerus followed by several courses of streptomycin. His course had been complicated by the development of a large cold abscess in the left axilla which had been treated unsuccessfully with streptomycin, immobilization, and frequent paracenteses over a period of six months. Just prior to starting enzyme therapy, the fluctuant, rounded, nontender mass measured 8 cm. in diameter, and the overlying skin was reddened and tense. Aspiration yielded 40 ml. of opaque, reddish brown, viscous fluid.

The lesion was initially treated by needle aspiration and irrigation with enzyme solutions. No antituberculous drugs were used. After five days, one of the needle tracts opened spontaneously, forming a small sinus which allowed adequate drainage and afforded a route through which, by means of small plastic catheters, the enzyme solutions could be instilled and the abscess cavity irrigated.

The patient received twelve enzyme treatments over a thirty-eight-day period. These treatments consisted of instillation of varying amounts of enzymes, ranging from 38,000 to 180,000 units of streptokinase and 30,000 to 100,000 units of streptodornase, contained in 20 to 50 ml. of saline. Each instillation of enzymes was followed after twenty minutes by extensive irrigation with saline.

One of the most striking features of the patient's course was the rapidity with which the cavity diminished in size. At the end of the first week its capacity was only 15 ml. After four weeks the capacity had been reduced to 5 ml.

At the beginning of the fifth week of therapy, it was found that, by pressing firmly over the posterior portion of the abscess, approximately 10 ml. of sanguineous fluid containing large amounts of necrotic debris could be extruded via the needle-tract sinus. After three

more applications of the enzymes and daily emptying of the new-found pocket by manual pressure, drainage from the sinus ceased and there was no palpable fluctuation.

The axillary area remained healed during a follow-up period of one year.

The period of time from onset of therapy to clinical healing in this case was five and one-half weeks. Cultures were positive for *M. tuberculosis* throughout the period of enzyme therapy, the last one being obtained just three days before drainage ceased. This would indicate that the elements of tissue repair were allowed to progress and effect healing by the process of enzymatic debridement of the abscess, even though tubercle bacilli were not eradicated from the area.

The syrupy exudate contained within the cold abscess was found to be rapidly thinned out *in vitro* by the streptococcal concentrates. This exudate was liquefied just as rapidly *in vitro* by purified beef testicular hyaluronidase. Furthermore, spectrophotometric analysis revealed that the exudate contained only trace amounts of desoxyribonucleic acid. Likewise, Feulgen stains (2) of the exudate were devoid of extracellular nucleoprotein. This viscous substrate was thus proved to be hyaluronic acid, probably emanating from the diseased synovial membranes of the shoulder joint. It was already known that the streptococcal concentrates contained significant amounts (up to 80 turbidity units per vial of streptokinase-streptodornase) of streptococcal hyaluronidase, which in this case proved to be quite advantageous.

During the course of enzyme therapy, this patient developed a serum anti-streptokinase level of 400 units per ml. Due to the fact that large amounts of streptokinase-streptodornase were used in the irrigation of the local area, it is probable that this antibody level did not significantly interfere with the local enzyme action.

Case 13: Pott's disease with psoas abscess. This patient, a 68-year-old white male, gave a history of tuberculous infection dating back fifty-eight years, when two draining sinuses appeared adjacent to the lower lumbar spine. Over the course of many years, these sinuses healed but were replaced by three draining sinuses about the left hip. During the twenty years prior to admission, these sinuses had continued to drain copious amounts of foul-smelling, thick, purulent material.

Initial roentgenographic examination revealed an extensive destructive lesion of the hip with dissolution of the femoral head and neck, and initial Lipiodol® studies showed that the sinus tracts led directly to the diseased bone. There was also free communication between the three sinuses, and enzyme treatment was given by inserting Foley catheters into the tracts, then filling the area with enzyme solution.[4] The enzymes were left in place for four to six hours and then washed out with saline. Although there were at times other organisms present, *B. proteus* predominated. During the course of treatment several cultures were positive for *M. tuberculosis*.

The full extent of the cavity which was being treated was not realized until a Diodrast® study was performed during the fifth week of therapy. It was found that 110 cc. of the radio-opaque material could be instilled into the area, and roentgenographic examination revealed an extensive psoas abscess extending from the level of the third lumbar vertebra to involve the hip joint. A collapsed second lumbar vertebra was presumed to be the source

[4] Although there was some variation in total dosage from day to day, each 50 ml. of the enzyme solution contained approximately 150,000 units of streptokinase and 75,000 units of streptodornase.

of the tuberculous abscess. It was apparent that the enzyme had permitted this exact definition of the diseased area by liquefaction of the contained exudate and necrotic debris, thus greatly increasing the size of the area accessible to topical therapy.

Because of persistently positive cultures for *B. proteus*, the patient received chlortetracycline and chloramphenicol orally for a ten-day period starting during the fifth week, as well as almost daily enzyme instillations. During the ensuing two weeks, cultures of the exudate showed progressively less growth, and the capacity of the area rapidly decreased. During the seventh week the three sinus tracts closed over and healed.

Five weeks after completion of enzyme treatment, the *B. proteus* infection recurred, with reopening of all of the sinus tracts. At the beginning of a second course of enzyme therapy, it was found that the patient had developed a serum anti-streptokinase titer of 1,000 units per ml. The usual doses of the enzymes were found to be almost completely neutralized by the exudate contained within the abscess cavity. Significant levels of streptokinase in the local area could be effected only if the cavity received preliminary washings with enzyme solutions, thus temporarily combining the majority of the local antibody. After this initial procedure, large doses of the enzymes were left in place for two to four hours, and were then replaced by fresh enzymes; and the process was repeated as often as three times daily. Despite the intensiveness of the treatment, there was no noticeable improvement after three weeks, either in the character and amount of the exudate or the population of *B. proteus*. The areas were then treated concomitantly for one week with chlortetracycline also administered locally. On this regimen two of the three tracts healed, and discharge from the third was diminished. Not until three additional weeks had elapsed and the local administration of penicillin had been added to the regimen did the remaining sinus stop draining and heal.

The patient was soon thereafter transferred to an institution for custodial care, and his subsequent course is unknown. It is likely that the local disease recurred. However, this patient is presented merely to point out that a problem of this kind, in which surgical treatment is not feasible, can be adequately managed in a conservative manner.

As in *Case 12*, a large proportion of the exudate was found to consist of hyaluronic acid, presumably produced by diseased synovial membranes bordering the abscess cavity.

This case also stresses the importance of the use of antimicrobial drugs in combination with streptokinase-streptodornase when secondary infection plays a predominant role.

Case 14: Tuberculous tenosynovitis. This patient was a 25-year-old white male who gave a ten-month history of pain and swelling of the left palm and wrist following trauma to the fifth digit. The area was moderately tender. "Rice bodies" were readily palpable within the ulnar bursa, and there was marked limitation of motion of the little finger. The remainder of the physical examination, including the chest roentgenogram, was normal.

The ulnar bursa was tapped on admission and 1 ml. of serous fluid was obtained, which later proved to be positive for *M. tuberculosis* on culture. Two milliliters of saline, containing 22,000 units of streptokinase and 14,000 units of streptodornase, were injected at this time.

During the next eight days the bursa was tapped four times, with the removal of scanty amounts of purulent material. Thereafter fluid could no longer be obtained. Two weeks after enzyme treatment, "rice bodies" were no longer palpable, the local swelling was subsiding, and the patient noted progressive return of motion to the little finger. The rapid disappearance of the "rice bodies," presumed to consist mainly of fibrin, was attributed to the action of streptokinase.

Follow-up one year later revealed that improvement of the hand had been progressive. However, the patient had been admitted to another hospital in the interim with active pulmonary tuberculosis.

DISCUSSION

The mechanisms involved in the response of suppurative tuberculous lesions to streptokinase and streptodornase are probably multiple; however, some of the principles of the mechanism of their action have been established (3). The enzyme preparations used had no detectable effect on the growth of tubercle bacilli on Dubos medium. The action of streptokinase and streptodornase on their specific substrates has been extensively described (4, 5). What bearing these actions have on the treatment of the tuberculous suppurative lesion is a matter of conjecture. Extracellular nucleoprotein has been found to be abundant in suppurative tuberculous exudates, as determined by Feulgen staining (2). Its rapid disappearance after contact with streptodornase, with striking thinning of the exudate, probably breaks down a barrier which would block further enzyme action on underlying substrates. Similarly, fibrin may also play the role of a barrier.

The action of the streptococcal concentrates on caseous material is not clearly understood. Small flecks of caseous matter examined by the Feulgen stain showed a very fine reticulum of nucleoprotein extending throughout the section, but the greater portion of the specimen took on a nonspecific stain. When small amounts of caseous material were incubated with enzymes in a saline solution, there was no apparent change in the state of this material after several hours of observation; if serum was added to the test tube in the presence of enzymes, however, the caseous material was slowly digested. Moreover, it has been clinically apparent that caseous material lining tuberculous ulcers disappeared during the course of enzyme therapy. This caseous matter was often dislodged by irrigation but, in cases treated with enzyme jelly alone, the caseous material disappeared spontaneously.

Another clinical observation has been the rapid replacement of soft, mushy dirty gray tuberculous granulomatous tissue lining the wall of the ulcer, over the course of a few days' enzyme therapy, by exuberant, healthy looking, red granulation tissue. The reason for this beneficial tissue reaction is obscure. Serial biopsies taken of the basal tissue of these nodes indicated that microscopic foci of caseation disappeared completely, but even in lesions which were rapidly approaching healing there remained small foci of epithelioid cells and multinuclear giant cells embedded in the exuberant granulation tissue. Microscopic tubercles remained but were surrounded by a wall of healthy tissue.

A property of the enzyme action which must be considered in the healing of the tuberculous ulcer is the transient local inflammatory reaction which is characteristically brought about. Application of the enzymes to a diseased area causes the outpouring of fresh viable phagocytes (6) as well as serum. When this reaction occurs within a closed space, a febrile reaction of varying degree often follows. In the case of the draining tuberculous lesion, however, this inflam-

matory reaction may be of great benefit in bringing viable leukocytes to the diseased area, affording phagocytosis of undesirable elements. The outpouring of serum may also bring forth immune elements necessary for the control of the infection, as well as native proteolytic enzymes which may further the beneficial effects.

It should be pointed out that the streptokinase-streptodornase preparations employed in this study contained other enzymes and proteins, some of which are not clearly defined. For this reason an exact interpretation of the effects of each enzyme component on the tuberculous process is not possible. Certainly the hyaluronidase content of the streptococcal concentrates appeared to be of definite clinical benefit in at least 2 of the cases (*Cases 12* and *13*) presented in this report.

In none of the patients presented above were significant toxic reactions noted. This was believed to have resulted from the fact that adequate drainage of the treated lesion was provided for in all cases, either by incision and drainage or by needle aspiration at appropriate intervals.

Finally, it is believed that the personal factor in treating tuberculous lesions with streptokinase-streptodornase is important. Each lesion must be treated on an individual basis. Frequency and duration, as well as outcome of therapy, will depend heavily upon careful daily observation of the lesion.

SUMMARY

Local treatment with streptococcal concentrates of streptokinase and streptodornase has effected healing of seventeen suppurative tuberculous lymph nodes. All of the treated nodes have remained healed over varying specified follow-up periods.

In addition, successful enzyme treatment is described in 3 cases representing other types of extrapulmonary suppurative tuberculosis.

Methods of application, local factors relating to healing, indications for concomitant antimicrobial therapy, and the mode of action of the enzymes in the tuberculous process are discussed.

SUMARIO

La Estreptoquinasa-Estreptodornasa en el Tratamiento Local de la Tuberculosis Extrapulmonar Supurante

El tratamiento local con concentrados estreptocócicos de estreptoquinasa y estreptodornasa ha efectuado la cicatrización de diecisiete ganglios linfáticos tuberculosos supurantes. Todos los ganglios tratados han permanecido curados durante varios períodos estipulados de observación subsiguiente.

Además, describese el éxito que acompañó a la encimoterapia en tres casos que representaban otras formas de tuberculosis supurativa extrapulmonar.

Se discuten los métodos de aplicación, los factores locales relacionados con la cicatrización, las indicaciones de la terapéutica antimicrobiana concomitante y el modo de actuar de las encimas en el proceso tuberculoso.

RESUME

Streptokinase-Streptodornase dans le traitement local de la tuberculose extra-pulmonaire suppurée

Le traitement local avec des extraits concentrés de streptokinase et de streptodornase a permis d'obtenir la cicatrisation dans dix-sept adénites tuberculeuses suppurées. La guéri-

son de tous les ganglions traités s'est maintenue pendant les diverses périodes d'observation mentionnées.

De plus, il est décrit un traitement enzymatique qui a donné de bons résultats dans trois cas représentant d'autres formes de tuberculose extra-pulmonaire suppurée.

Les méthodes d'administration, les facteurs locaux intéressés dans le processus de cicatrisation, les indications du traitement antibactérien concomitant et le mode d'action des enzymes dans le processus tuberculeux sont commentés.

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STUDIES ON THE INTRATHECAL USE OF STREPTOKINASE-STREPTODORNASE IN TUBERCULOUS AND OTHER TYPES OF BACTERIAL MENINGITIS[1, 2, 3]

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Introduction

The observations recorded in this article deal with the use of streptococcal concentrates of streptokinase and streptodornase[4] administered intrathecally. Beneficial effects have been attributed to the intrathecal use of streptokinase in tuberculous meningitis in children by Cathie (1) and Cathie and MacFarlane (2) in England and by Williams and Bazeley (3) in Australia. These writers, as well as Giraud and Espinas (4) in France, reported minimal toxic effects from the intrathecal injections. However, Lorber (5) and Eadie (6), in presumably similar series of cases, could find no significant differences in results between the streptokinase-treated and control patients, and both reported moderate to severe toxic reactions in several instances following intrathecal instillation of streptokinase. Naismith (7) also reported serious toxic reactions.

Aron and Lecomte (8) described a severe toxic reaction to two intrathecal injections of streptokinase in a 33-year-old woman with tuberculous meningitis. The patient developed fever, severe back pain, and signs of meningeal irritation a few hours after each injection; and the cerebrospinal fluid at that time was described as xanthochromic, under increased pressure, and contained many erythrocytes and leukocytes. The cerebrospinal fluid findings reverted to the pre-injection state within fifteen days after each injection.

Hedlund and associates (9) in Sweden used streptokinase in combination with streptomycin as a prophylactic measure in preventing subarachnoid block in a small series of patients with tuberculous meningitis. Subarachnoid blocks formed during this treatment in two of three patients. Lysis of existing block was claimed in two additional cases. No mention was made of toxic effect.

It should be pointed out that the streptokinase preparations employed by the above investigators were prepared independently by methods dissimilar to those

[1] From the Department of Medicine, New York University College of Medicine, and the Third Medical Division of Bellevue Hospital, New York, New York.

[2] This study was supported through a contract with the Medical Research and Development Board of the Office of the Surgeon General, Department of the Army, U. S. A.

[3] After preparation of this manuscript, studies on the mechanics of streptokinase activity in tuberculous meningitis were reported by Fletcher (J. Clin. Investigation, 1954, *33*, 69). This investigator has carried out quantitative studies of the streptokinase-activated fibrinolytic system *in vivo* in patients with tuberculous meningitis, with special reference to the changes in plasminogen concentrations and the appearance of a plasmin inhibitor in the cerebrospinal fluid following instillation of small amounts of streptokinase intrathecally.

[4] Supplied by Lederle Laboratories under the trade name "Varidase."

used in this country and, therefore, may not be comparable with the enzyme preparations used in this investigation.

The purposes of the present study were: (*1*) to determine local or systemic toxicity of streptokinase-streptodornase; (*2*) to make quantitative estimations of the lytic system in the cerebrospinal fluid following the injections; (*3*) to utilize the two enzymes clinically, as therapeutic agents, in patients with meningitis.

The patients to be reported consisted of: 6 subjects with normal cerebrospinal fluids; 6 patients with tuberculous meningitis; 4 patients with acute purulent meningitis, 3 caused by pneumococcus and one of undetermined etiology; and one patient with meningitis due to *Pseudomonas aeruginosa*.

Materials and Methods

In the tabulated data pertaining to the cases presented below, plasminogen, plasmin, and streptodornase were measured in the cerebrospinal fluid at various specified intervals following the intrathecal injection of the streptococcal concentrates.

Plasminogen (*activatable fibrin-lysing system*) in the cerebrospinal fluid and streptokinase content of the enzyme preparations injected were measured according to the methods of Christensen (10).

Plasmin (*active fibrin-lysing system*) was measured by adding 0.1 ml. of cerebrospinal fluid to Christensen's standard fibrin clot (10), to which no plasminogen had been added. Results were expressed as 0 to 4-plus, depending upon the length of time required for lysis, as follows:

Lysis up to 1 hour	4+
Lysis 1–6 hours	3+
Lysis 6–24 hours	2+
Lysis 24–48 hours	1+
Lysis 48–72 hours but no lysis in controls	trace
No lysis	0

Streptodornase in the cerebrospinal fluid was measured on a few occasions by the viscosimetric technique described by Christensen (10). In most instances, however, the presence of streptodornase was determined by an alcohol precipitation technique similar to that used by McCarty (11) for antibody determinations. With this technique, 0.4 ml. of cerebrospinal fluid was mixed with 0.6 ml. of a 0.15 per cent solution of highly polymerized sodium desoxyribonucleate and incubation at 30°C. was carried out for thirty minutes. If, upon the addition of 1 ml. of alcohol, no characteristic fibrous white precipitate formed, the amount of streptodornase was deemed adequate.

On occasion, anti-streptokinase levels were determined on both serum and cerebrospinal fluid, according to the method of Christensen (10).

It should be emphasized that the streptococcal concentrates rich in streptokinase-streptodornase also contained other known and unknown enzymes and proteins (12). Consequently, it is clearly recognized that the determination of the intrathecal reactions with respect to a specific enzyme must await the availability of the individual enzymes in a highly purified form. For the purposes of

this study, quantitative references are made only to streptokinase and streptodornase.

Results

Effect of streptokinase-streptodornase intrathecally in normal human subjects

In a preliminary study designed to evaluate the toxicity of the enzymes, 6 subjects with normal cerebrospinal fluid were given small intrathecal doses. The first normal subject was given 20 units of streptokinase and 4 units of streptodornase, an amount equal to 1/10,000 of the usual dosage given intrapleurally to patients with hemothorax and empyema (13). The patient developed no symptoms referable to the injection, and there were no detectable changes in the cerebrospinal fluid.

The next 5 control subjects were injected with approximately 200 units of streptokinase and 40 units of streptodornase. The results are summarized in

TABLE 1

Data on Six Normal Subjects Receiving Intrathecal Streptokinase(SK)-Streptodornase(SD)

Case Number	Age	SK-SD Administered	Cerebrospinal Fluid Findings:			
			Time After Injection	Erythrocytes	Leukocytes	Plasminogen
	years	*units*		*per cu. mm.*	*per cu. mm.*	
1	60	SK-20 SD-4	24 hours	0	0	2 units per ml.
2	65	SK-220	0	0	0	2 units per ml.
		SD-30	24 hours	580	9,185	32
			72 hours	0	225	16
			9 days	8	4	—
3	3½	SK-220	0	0	0	2 units per ml.
		SD-140	24 hours	1,340	1,580	8
			72 hours	traumatic tap; xanthochromic.		supernatant 20
4	40	SK-240	0	0	3	4 units per ml.
		SD-50	24 hours	180	7,500	64
			48 hours	1,270	2,167	64
			72 hours	0	750	32
			7 days	0	500	16
			10 days	0	340	16
			17 days	0	65	16
5	72	SK-240	0	0	0	4 units per ml.
		SD-50	4 hours	2,600	80	64
			24 hours	2,950	6,050	—
			72 hours	0	540	—
6	2	SK-160	0	0	0	—
		SD-34	5 hours	110	7,000	—
		heated ½ hour at 56°C.	24 hours	130	1,140	—
			72 hours	5	310	—

table 1. Each person exhibited a toxic reaction to the enzymes; some reactions were more marked than others. The clinical symptomatology varied from a mild febrile response without other signs in *Case 3* to headache, nausea, vomiting, meningeal signs, and fever up to 104°F. in *Case 5*. In general, the initial symptoms occurred within four to six hours, reached their peak at eight to twelve hours, and disappeared within two to five days. The cerebrospinal fluid findings in all of the 5 patients included a rise in pressure (to 360 mm. in *Case 5*), appearance of abundant leukocytes (polymorphonuclear leukocytes) and erythrocytes, and limited increases in plasminogen concentrations, all subsiding within three to six days. It is noteworthy that, during the three to four days after injection of the enzymes, a small pellicle was frequently found in the cerebrospinal fluid specimens on standing.

One of the normal group, *Case 6*, was injected with 1 ml. of a saline solution containing 160 units of streptokinase and 34 units of streptodornase which had been heated for thirty minutes at 56°C. This procedure destroyed approximately 95 per cent of the streptodornase and approximately half of the streptokinase so that there were approximately 80 units of streptokinase and less than 2 units of streptodornase present. In spite of the virtual removal of streptodornase, a cellular reaction of considerable magnitude occurred. Streptodornase alone, therefore, is probably not necessary for the production of the tissue reaction.

Laboratory determinations: The normal concentration of plasminogen in cerebrospinal fluid has been found to be very low, usually between 2 and 4 units per ml., as compared to normal serum values of about 7,000 units per ml. (14). Furthermore, the amount of plasminogen in the cerebrospinal fluid usually varies with the amount of protein present. In all of the normal subjects in whom plasminogen concentrations were determined, there was a post-injection rise. This was thought to be due to exudation brought about by enzyme instillation.

The complete streptococcal fibrin-lysing system (plasmin) set up in the cerebrospinal fluid of these normal subjects was not measurable at this streptokinase dosage.

The appearance of erythrocytes in the cerebrospinal fluid with subsequent xanthochromia was thought at first to be the result of traumatic spinal taps. However, it has proved to be a constant finding in post-injection spinal taps in patients, the mechanism of which is not yet understood.

EFFECT OF STREPTOKINASE AND STREPTODORNASE IN PATIENTS WITH TUBERCULOUS MENINGITIS

In initiating the study of the effect of enzymes on patients with tuberculous meningitis, it was decided to start with 5,000 units of streptokinase and 1,000 units of streptodornase. This amount was calculated to achieve the same dilution of enzymes in the cerebrospinal fluid as had been used intrapleurally with pleural fluid in the treatment of thoracic conditions (13). As may be noted in subsequent tables, however, the amount of enzymes administered has been substantially increased. The factors relating to dosage will be considered later.

Group A

Group A consisted of 3 children who were treated on the Children's Chest Service of Bellevue Hospital. Two of these, B. P. and J. R., experienced relapses of tuberculous meningitis on several occasions; the third, P. G., was a six-month-old baby in whom a spinal subarachnoid block was demonstrated on admission. In addition to enzyme therapy described below, all were being treated with streptomycin and sulfone (Promizole®) according to the methods of Lincoln and Kirmse (15).

Case 1: Patient B. P., six years old, had been under intensive treatment for tuberculous meningitis for two years and the infection had relapsed on three occasions. Shortly after the third relapse, streptokinase-streptodornase was given for a total of six injections over a three-week period. Subsequent reports of cerebrospinal fluid cultures were negative for *M. tuberculosis* just prior to, during, and for five months following enzyme treatment.

Febrile reactions were not excessive during enzyme therapy, with rises to 103°F. on two occasions, despite the fact that the patient received ten to twenty times as much enzymes as had the normal subjects. In general this patient's total clinical reactions were considered minimal. Following enzyme therapy the patient improved rapidly and the cerebrospinal fluid showed steady improvement in that sugar and protein returned toward normal values.

The patient was clinically well for a six-month period when a fourth relapse occurred, confirmed by the isolation of *M. tuberculosis* from the cerebrospinal fluid. Intercurrent infection supervened, and the patient died two weeks later of a fulminating *Streptococcus fecalis* meningitis.

The laboratory data obtained from this patient are summarized in table 2.

In all instances, five hours after the intrathecal injection of the enzymes in this patient, there was: (*1*) a fall in the concentration of plasminogen; and (*2*) demonstra-

TABLE 2

ENZYMATIC COMPONENTS MEASURED IN CEREBROSPINAL FLUID FIVE HOURS AFTER INTRATHECAL INJECTION OF STREPTOKINASE(SK)-STREPTODORNASE(SD)

Patient B. P.

Treatment Number	Units of SK-SD Administered	Plasminogen (Units per Ml.)		Plasmin	SD (Units per Ml.)
		(Pre-injection)	(Post-injection)		
1	SK 5,000 SD 1,000	16	2	3+	—
2	SK 12,000 SD 3,000	16	2	2+	—
3	SK 18,000 SD 4,500	16	8	4+	—
4	SK 18,000 SD 4,500	32	8	4+	12
5	SK 21,600 SD 5,400	32	4	3+	9
6	SK 21,600 SD 5,400	—	—	2+	—

ble fibrin-lysing activity (plasmin). Streptodornase was measured on two occasions and found to be present. The cellular responses are not tabulated, but there was a pre-injection average leukocyte count of 350 per cu. mm. and a five-hour post-injection average leukocyte count of 1,450 per cu. mm., with a rise in percentage of polymorphonuclear cells from 30 per cent before injection to 80 per cent after injection. The post-injection rise in erythrocyte count varied from 300 to 2,000 per cu. mm.

Case 2. Patient J. R., five years old, had sustained two clinical relapses of tuberculous meningitis over a period of a year and a half. As in the case of the patient just described, he received a course of six intrathecal injections of streptokinase-streptodornase over a three-week period, and the amount of enzymes administered was the same for both patients. His response to enzyme instillation was similar to that of *Case 1*, in regard both to the clinical reaction and the laboratory data obtained. Cultures of the cerebrospinal fluid were positive for *M. tuberculosis* before, during, and after the period of enzyme therapy. Moreover, the patient's disease progressed steadily, and he died two months after the completion of enzyme therapy.

Case 3. Patient P. G., six months old on admission, was found to have a spinal subarachnoid block with yellow fluid of high protein content (1.3 gm. per 100 ml.) in the lumbar theca. In attempting to cause lysis of the block, the first dose of streptokinase-streptodornase, given via the lumbar route, was without apparent effect. In approaching the block from above, two injections of the enzymes were given via the cisterna magna in forty-eight hours. Five hours after the second injection, definite evidence for lysis of the block was obtained in that an abundance of fluid collected from the lumbar region was shown to contain plasmin, and the physical and chemical characteristics of the cisternal and lumbar fluids were identical.

Despite continuation of enzyme therapy in an effort to maintain patency of the subarachnoid space, the block recurred after six days. Repeated attempts to lyse the block by instillation of enzymes into the cisterna magna were unsuccessful, and at the end of two weeks cisternal fluid could no longer be obtained. During this period it was found that no plasmin could be demonstrated in the cisternal fluid five hours after injection, although streptodornase was present in measurable quantity.

In vitro studies revealed the appearance in the cerebrospinal fluid of an inhibitor to streptokinase which could be overcome by the supplementary use of normal human serum as a source of plasminogen, which was apparently lacking in the cerebrospinal fluid.[5] Accordingly, on the thirty-second day of treatment following insertion of a tube into the right lateral ventricle, 25,000 units of streptokinase mixed just prior to injection with 5 ml. of normal human serum were instilled into the ventricle. Fluid removed thirty minutes later revealed only a trace of plasmin.

On the thirty-sixth day of treatment, in an effort to set up an effective fibrinolytic system, large amounts of streptokinase and serum were given at frequent intervals during the course of the day. The fibrinolytic activity resulting from this procedure is presented graphically in figure 1.

[5] The apparent low concentration of plasminogen, in spite of an increase in cerebrospinal fluid protein, was later found to be erroneous and was due to the large amount of streptokinase inhibitor present in the tested cerebrospinal fluid which immediately inactivated the standard amount of streptokinase used in the test for plasminogen. When larger amounts of streptokinase were employed in the test, the plasminogen concentration was found to be approximately 80 units per ml.

It may be seen (figure 1) that a very active fibrin-lysing system (4-plus plasmin) was maintained for several hours, and it was present in small amounts (1-plus plasmin) the next morning, fifteen hours after the last injection of streptokinase and serum. It is of interest that, once the active system was set in motion in this patient, the amount of fibrinolytic activity was increased by the addition of either enzyme alone or serum alone. Further *in vitro* studies identified the inhibitor in the cerebrospinal fluid as the specific antibody, anti-streptokinase, which was also found in high concentration in the patient's serum. The above-described method for overcoming this antibody was discovered too late, as the block, undisturbed for a month, had probably become organized. During the ensuing month the patient developed signs of hydrocephalus and died.

As in the other 2 cases in this group, toxic manifestations, as reflected in febrile and cellular responses following the use of streptokinase-streptodornase in this patient, proved to be minimal although large doses of the enzymes as well as serum were employed.

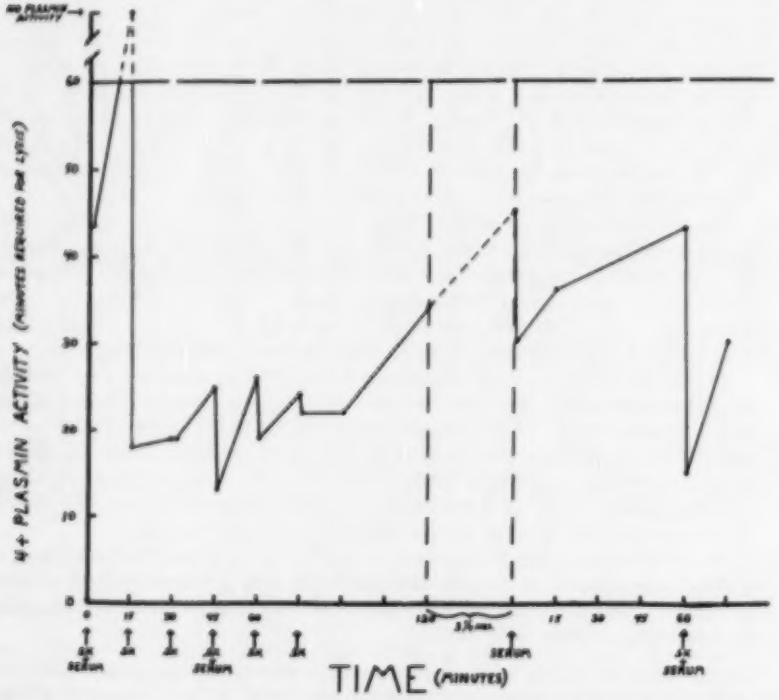

FIG. 1. Plasmin determinations, ventricular fluid (patient P. G.) showing response to streptokinase plus serum, streptokinase alone, and serum alone (streptokinase—125,000 units; serum—5 ml.).

Group B

Group B consisted of 3 cases of tuberculous meningitis which differed from the above pediatric cases in one main respect: the tissue reaction to the intrathecal instillation of enzymes was quite severe. This difference was attributed to the fact that the patients in this group were twelve, sixteen, and forty-eight years old as opposed to ages five, six, and six months in *Group A*. As far as could be ascertained, the age difference seemed to be the only significant difference between the two groups, although there was a striking difference in these patients' reactivity to the intrathecal administration of streptokinase-streptodornase.

Case 4. Patient M. K., twelve years old, was admitted with a three-week history of lethargy, headache, and vomiting, and on physical examination showed hypoactive reflexes and nuchal rigidity. During the first three weeks seven cerebrospinal cultures were positive for tubercle bacilli. The patient was started on intrathecal and intramuscular streptomycin as well as sulfone therapy.

A summary of enzyme treatments given and data obtained are presented in table 3.

Following the first injection of enzymes, the temperature rose to 103°F. and the

TABLE 3

SUMMARIZED DATA ON PATIENT M. K. (*Case 4*)

Treatment Number	Day of Treatment	SK-SD Administered (in 1,000's of Units)	Time After Injection Tap Performed	Leukocytes	Cerebrospinal Fluid Pressure	Plasminogen Units per Ml.	Plasmin
				per cu. mm.			
1	First	SK 10 SD 2.5	0	530	300	16	—
			5 hours	2,500	—	16	2+
2	Fourth	SK 20	0	900	300	16	—
		SD 5	5 hours	4,700	400	64	0
3	Eighth	SK 30	0	570	400	16	—
		SD 7.5	5 minutes	—	—	—	4+
		+5 ml. of serum	5 hours	8,300 large coagulum	More than 600	128	2+
4	Twenty-second	SK 30	0	180 (pellicle)	300	32	—
		SD 7.5	1 hour	140 (no pellicle)	150	8	4+
			24 hours	9,350	(Block*)	64	0
			48 hours	650	410	32	—
5	Fifty-second	SK 15	0	550	130	64	—
		SD 6	5 minutes	—	—	—	4+
			4 hours	large coagulum	(Block*)	640	0
			18 hours	25,000 (gel)	170	2,560	—
			5 days	600	150	128	—

* *See* text

patient complained of severe headache and exhibited marked nuchal rigidity. The cellular response was moderate, but there was very little plasmin in the five-hour specimen. Following the second injection, which was twice the amount of the first, there was no detectable enzyme system, and the plasminogen concentration, instead of falling, rose. Although the febrile response was completely blocked by the use of amidopyrine, the local reaction was more severe, as manifested by the outpouring of cells and serum. After five hours, a large pellicle, presumed to be fibrin, formed immediately upon removal of the fluid from the intrathecal space.

The amount of enzymes was increased on the third and fourth injections and 5 ml. of normal human serum were added as a source of plasminogen. Five hours after the third injection, measurable amounts of the fibrinolytic system were present in the spinal fluid. A large pellicle formed in this specimen and subsequently disappeared after twelve hours' incubation at 37°C. Inasmuch as there was no pellicle in the cerebrospinal fluid removed the next day, it seemed probable that the relatively slow but persistent action of the fibrinolytic system had accounted for the excess fibrinogen presumed to be present in the cerebrospinal fluid.

One hour after the fourth injection there was no physical change in the cerebrospinal fluid, plasmin and streptodornase were abundant in quantity, and the plasminogen concentration had fallen. The next day, however, the ensuing tissue reaction had brought about a considerable increase in leukocytes and a rise in plasminogen to 64 units per ml., but no pellicle formation.

Clinical manifestations of the tissue reaction following the third and fourth injections were: (*1*) rise in temperature within eight hours, reaching a peak of between 103° and 104°F., and return to pre-injection levels by the next day; (*2*) increased nuchal rigidity for two days following each injection; and (*3*) severe frontal and occipital headaches. Twenty-four hours after each of these injections there was a definite spinal subarachnoid block as determined by manometrics. This block may have been due to edema of the local meninges as a part of the inflammatory reaction. It was a transient block which disappeared within two days as other indices of inflammation showed regression, i.e., temperature, cells, and increased plasminogen.

The reaction following the fifth injection of enzymes was particularly serious, although the amount of enzymes administered was relatively small and no serum was added to it. Four hours after the injection the temperature had risen to 103°F. and a lumbar puncture performed at this time produced markedly xanthochromic fluid containing many erythrocytes, and a large coagulum developed on standing. Plasminogen had risen from 64 to 640 units per ml. and the highly active fibrinlysing system present in the cerebrospinal fluid five minutes after injection had disappeared. Manometric block was present.

Ten hours after injection the patient lapsed into coma, had several convulsions, and the temperature was recorded as 108°F. Vigorous alcohol sponging caused a rapid fall in temperature, which returned to preinjection levels by the next day and remained so. The patient remained comatose for two days, followed by gradual and steady improvement with complete subsidence of the reaction within five days.

At a spinal tap performed seventeen hours after the injection, the cerebrospinal fluid pressure was recorded as 170 mm., the fluid did not coagulate, and there was no evidence of block; but the cloudy, yellow, serum-like fluid removed contained many erythrocytes and 25,000 leukocytes per cu. mm.; the plasminogen was found to be 2,560 units per ml. at this time. The plasminogen concentration on the following day

was reduced to 160, and five days after the reaction the cerebrospinal fluid findings had reached the pre-injection state.

During the following month the patient improved rapidly both clinically and in respect to cerebrospinal fluid findings. Two and one-half months after admission the patient was clinically well. She was entering the last week of her six months' course of intramuscular streptomycin when a relapse of her disease occurred. Streptokinase-streptodornase was not employed because of the previous experience in this patient. In spite of intensive streptomycin therapy, the patient died two months following the onset of her final relapse, with evidence of subarachnoid block and hydrocephalus.

The reason for the extreme severity of this patient's reaction to the last injection of enzymes is not clear. It was speculated that hypersensitivity to the enzymes may have developed following repeated injections. No correlation could be observed, however, between this reaction and sensitivity to the enzymes as determined by intracutaneous skin testing, which proved to be negative. Furthermore, hypersensitivity has never been observed in several hundred patients treated with streptokinase-streptodornase in this clinic. The toxic reaction in this patient may have been similar to that reported by Craddock and Haddock-Suarez (16). In their patient, a 42-year-old man with tuberculous meningitis, marked hyperthermia (108°F.) with a fatal termination occurred after the intrathecal injection of streptokinase-streptodornase. However, the patient was gravely ill and in a semi-comatose state prior to enzyme therapy.

The remaining two patients in *Group B* were subjects with tuberculous meningitis who presented the picture of spinal subarachnoid block to whom the enzymes were given solely in an effort to bring about lysis of these blocks. No quantitative studies of enzyme systems were carried out, but certain features of their clinical courses and results of treatment are recorded.

Case 5. Patient M. G., sixteen years of age, was found to have a spinal subarachnoid block on admission. The first injection of enzymes (streptokinase, 14,000 units-streptodornase, 5,000 units) was instilled into the lumbar theca. Within five hours the temperature rose from 101° to 103°F. and the patient complained of severe pains in the lumbar region radiating down the thighs. After twelve hours, the back pain subsided and was replaced by headache. Manometric evidence of persistent block remained and lumbar fluid obtained twenty-four hours after injection was cloudy and yellow and produced a large coagulum on standing.

Another unsuccessful attempt to eradicate the subarachnoid block was made by instilling 3,500 units of streptokinase and 1,500 units of streptodornase into the cisterna magna. The toxic reaction was severe. Ten minutes after injection the patient complained of sudden pain in the back of the neck at the injection site. After two hours the neck pain was replaced by a generalized headache, and the patient complained of marked dizziness and retched almost continuously for two hours. Five hours after injection, cloudy yellow fluid was removed from the cistern in which a large coagulum formed a few minutes after removal. Headache and lethargy persisted for five days, during which time opisthotonus was marked. A cisternal tap one

week later revealed fluid normal in every respect in which no cells were seen. Repeated lumbar punctures were unproductive.

The patient's clinical course following enzyme therapy remained stationary for six weeks, at which time he was transferred to a sanatorium. His further course is unknown.

Case 6. Patient M. J., forty-eight years old, had tuberculous meningitis complicated by a spinal subarachnoid block which developed at the end of two weeks of intrathecal streptomycin therapy. The lumbar fluid was rich in protein and cells, whereas the cisternal fluid showed near-normal values.

It was decided to attempt lysis of this block by flushing the area with enzyme solution until there was definite manometric evidence for lysis. Accordingly, the patient received rigorous treatment on two occasions eight days apart. The technique consisted of repeatedly removing 10 ml. portions of cerebrospinal fluid and slowly replacing this amount with enzyme solution. During the first treatment, manometric evidence for lysis of the block was obtained within twenty-five minutes. However, within four hours a toxic reaction of considerable magnitude occurred, with reappearance of the block. During the second treatment, in which 300,000 units of streptokinase were employed, manometric evidence for patency of the spinal subarachnoid space did not occur until irrigation had been employed for two and one-half hours. Two hours later there was a severe reaction to the enzymes with recurrence of block. Attempts to prevent the toxic response by replacing enzyme-containing cerebrospinal fluid with large amounts of saline were of no avail. On the following day the patient was semi-comatose, and nuchal rigidity was marked. A cisternal tap at that time revealed highly cellular yellowish fluid which developed a large pellicle on standing, further evidence that lysis of the block had been accomplished on the preceding day, only to recur as a result of the overwhelming irritative reaction.

The patient appeared to be doing well despite the subarachnoid block when, six weeks after administration of enzymes, he died suddenly and, as no autopsy was done, the immediate cause of death remains obscure.

EFFECT OF STREPTOKINASE-STREPTODORNASE IN 4 PATIENTS WITH ACUTE PURULENT MENINGITIS

The intrathecal use of streptokinase-streptodornase in acute types of meningitis has not been extensive, but the results obtained in 4 treated cases presented briefly below have been uniformly good. The toxic reactions following the intrathecal administration of the streptococcal concentrates into patients with acute purulent meningitis have been negligible.

This observation is in accord with the findings of French investigators in two cases of acute bacterial meningitis treated with streptokinase-streptodornase in conjunction with antimicrobial drugs. Milliez, Mayer, and Levasseur (17) reported striking dissolution of a spinal subarachnoid block due to purulent exudate in a case of staphylococcal meningitis. Lievre and associates (18), in another case of staphylococcal meningitis, reported rapid thinning of a thick purulent cerebrospinal fluid. Both of these patients were adults in whom the use of the enzymes was unaccompanied by any manifestations of toxicity and was followed by rapid sterilization of the cerebrospinal fluid. In this country,

Trice and Townsend (19) reported the use of streptokinase-streptodornase in an infant with meningitis due to *Klebsiella pneumoniae*. Although the patient eventually died of his disease, these writers noted no toxic effect from the intraventricular instillation of large doses of streptokinase-streptodornase and concluded that enzyme therapy had been of some benefit in retarding the development of hydrocephalus.

Case 7. Patient K. J.,[6] a five-month-old infant with pneumococcal meningitis, was treated for five days with large doses of parenteral penicillin with slight improvement in the clinical picture as well as in the cerebrospinal fluid findings. However, there was persistence of a low-grade fever, marked stiffness of the back and neck, and visible bulging of the anterior fontanelle. On the fifth day 10,000 units of streptokinase and 2,000 units of streptodornase were instilled into the lumbar theca. Manifestations of toxicity were completely absent but there was no definite clinical improvement. Three days later another intrathecal injection was given, containing 16,000 units of streptokinase and 4,000 units of streptodornase. Cerebrospinal fluid obtained five hours later was found to contain measurable amounts of plasmin and streptodornase, with a fall in the concentration of plasminogen. There was no rise in temperature. Thereafter clinical improvement was rapid, and one week following the last injection of streptokinase-streptodornase physical examination was normal in every respect. The baby was observed for a year and appeared to be entirely normal.

In this patient the intrathecal administration of streptokinase-streptodornase was completely devoid of demonstrable toxic effect.

Case 8. Patient G. K.,[7] three weeks old on admission, was semicomatose with twitchings of the face and left extremities. The anterior fontanelle was visibly bulging, with spreading of all sutures. Lumbar fluid was thick and turbid, was withdrawn with considerable difficulty, and gelled immediately upon removal. There were 7,000 leukocytes per cu. mm., most of which were polymorphonuclear leukocytes. Possibly due to a course of penicillin therapy prior to admission, no organisms were recovered from the spinal fluid.

During a five-day period the baby received, in addition to antimicrobial drugs, four intrathecal injections, each containing 16,000 units of streptokinase and 8,000 units of streptodornase. The first injection was given via the cisterna magna because fluid could not be obtained from the lumbar area. The cisternal fluid was very thick, comparable to that removed by lumbar puncture on admission. Three hours after instillation of the enzymes the cisternal fluid was thin, was readily removed, and showed no clot on standing. The next day, thin fluid was readily obtainable from the lumbar region. Three further injections of the enzymes were given without noticeable change in the physical character of the cerebrospinal fluid. Following one of the enzyme treatments, the temperature rose to 102°F., but no temperature rise occurred after the other injections. Following this five-day regimen the patient improved rapidly and was subsequently discharged on the twenty-first day. On follow-up two months later, physical examination was entirely normal.

[6] Treated at Gouverneur Hospital, New York City.

[7] Treated at Brooklyn Jewish Hospital.

In this patient the enzymes caused a marked thinning of the thick purulent cerebrospinal fluid which promoted aspiration and possibly allowed the antimicrobials, through greater diffusion, to be more effective in eliminating the causative organisms. It is of special significance that the use of the enzymes was not accompanied by a toxic reaction even though they were instilled directly into the cisterna magna.

Case 9. Patient J. G., forty-eight years old, developed pneumococcal meningitis (type II pneumococcus) a few days following the onset of pneumococcal pneumonia. He was responding to parenteral penicillin and sulfadiazine therapy when he received his first dose of enzymes. The enzyme therapy was started on the third day following onset of the meningitis, by which time his temperature had fallen from 104° to 102°F., and the cerebrospinal fluid, although still highly cellular, reflected improvement. The first injection of enzymes consisted of 20,000 units of streptokinase and 6,000 units of streptodornase, administered via the lumbar route. A tap performed five hours later revealed no rise in cerebrospinal fluid pressure, a moderate rise in leukocytes from 500 to 2,000 per cu. mm., a fall in plasminogen from 32 to 2 units per ml., and measurable concentrations of plasmin. His temperature remained at the pre-injection level of 102°F. following this injection, and the patient, fully conscious, offered no new complaints.

The next day 30,000 units of streptokinase and 12,000 units of streptodornase were administered, contained in 5 ml. of normal human serum. Results again were optimal. A tap done sixteen hours after injection showed a slight fall in pressure, a fall in plasminogen from 16 to 4 units per ml., and a slight rise in leukocytes from 410 to 750 per cu. mm. The post-injection temperature remained at 101°F. The patient's rapid recovery from his disease was uneventful. He was followed for several months, and at no time were there any clinical signs of meningeal residua.

In this case it was clearly evident that the intrathecal instillation of the enzymes had no toxic effect in spite of the fact that serum, which was associated with severe reaction in one of the cases of tuberculous meningitis (*Case 4*), had been employed.

Case 10. Patient S. T., eight months old, had been ill for two weeks prior to admission, was stuporous, and exhibited weakness of the right extremities, unequal pupils, and a right internal strabismus. Cerebrospinal fluid culture showed a pneumococcus. After one week of intensive treatment with antimicrobial drugs, the clinical picture did not change, although the cerebrospinal fluid became sterile and showed fewer cells. Enzyme therapy was employed in an effort to lyse any localized exudations which were believed to be responsible for the neurologic changes by reason of their close proximity to vital neurologic centers. The first injection consisted of 16,000 units of streptokinase and 5,000 units of streptodornase. A one-degree rise in temperature six hours after the injection was the only manifestation of toxicity. The neurologic abnormalities persisted. Four days later, streptokinase (30,000 units) and streptodornase (10,000 units) were administered. After five hours it was noted that the strabismus and right-sided weakness, as well as the inequality of the pupils, were no longer present, and the patient remained neurologically normal until discharge three weeks later. The patient was re-examined two months after discharge, at which time no physical abnormalities could be detected.

The striking change in the neurologic picture appeared following the second enzyme injection and is presumed to have resulted from effective lysis of the residual exudate. An additional finding of interest was derived from this patient. A specimen of ventricular fluid obtained four hours after the injection of enzymes into the lumbar theca was found to contain measurable amounts of plasmin and streptodornase. This indicated that the enzymes are effectively dispersed throughout the cerebrospinal fluid system and may be operative at sites distant from the site of injection when no block is present.

EFFECT OF STREPTOKINASE-STREPTODORNASE IN A PATIENT WITH 'PYOCYANEUS' MENINGITIS

Patient W. G., forty-one years old, had a chronic type of meningitis due to *Pseudomonas aeruginosa* which had been treated unsuccessfully for a period of seven months with a large variety of antimicrobial drugs. The organism was quickly eradicated from the cerebrospinal fluid by a single intrathecal injection containing 12,000 units of streptokinase and 3,000 units of streptodornase.

This case has been presented briefly elsewhere (12), but certain features of the patient's reaction to the enzymes are recorded here. A tap performed twenty-four hours after the injection revealed homogeneously pink, turbid fluid under increased pressure (400 mm.), containing 35,000 erythrocytes and 4,500 leukocytes per cu. mm., with increased plasminogen. Within thirty minutes after the injection, the patient complained of frontal and occipital headache. Temperature rose to a level of 103°F. during the day after injection, with rapid lysis of fever on the third day. The patient vomited several times during the evening following injection and could remember nothing of the first eighteen hours of the reaction save an intensely severe headache. On the second day, definite nuchal rigidity, as well as positive Kernig sign, was noted.

The signs and symptoms as well as the above-described cerebrospinal fluid findings subsided within four days except for headache, which persisted for two months. Six months after the injection of enzymes, the cerebrospinal fluid still showed 45 leukocytes per cu. mm., but at that time and again six months later the patient was asymptomatic and physical examination was completely normal.

As a contributing factor to the rapid sterilization of the cerebrospinal fluid, it seems likely that the organisms were engulfed by the abundance of fresh cells which poured into the fluid as a result of the cellular reaction to the enzymes.

Knight, Hardy, and Negrin (20) have reported successful treatment of a patient with chronic meningitis due to *Ps. aeruginosa* with streptokinase-streptodornase in conjunction with neomycin. Reactions to the intrathecal injection of the enzymes were also of severe degree in their patient.

Discussion

The main problem in the intrathecal use of streptokinase-streptodornase has been that of toxicity, the basic cause of which is not known. However, an analysis of this toxic reaction gives some degree of insight into its general nature.

The *severe reaction* has occurred in the control normal subjects; the older patients with tuberculous meningitis (*Group B*); and the patient with meningitis due to *Ps. aeruginosa*.

The reaction was characterized clinically by fever of varying degree, and the occurrence (also in varying degree) of headache, nausea and vomiting, increased nuchal rigidity, increased meningeal signs and, in one instance, coma. Symptoms usually occurred within five hours after the intrathecal injection and in each instance were transient without detectable residual changes. The local irritative effect was characterized by an outpouring of large numbers of leukocytes and erythrocytes into the cerebrospinal fluid; an increase in cerebrospinal fluid pressure, sometimes marked; an increase in protein including plasminogen, with frequent pellicle formation in cerebrospinal fluid specimens; and usually a complete absence of enzymes instilled five hours previously, presumably due to the outpouring of inhibiting substances from the serum.

The non-toxic or *optimal reaction* has occurred in the children with tuberculous meningitis (*Group A*) and the patients with acute types of meningitis due to pyogenic bacteria.

In these patients the reaction consisted of very few post-injection symptoms and little or no rise in temperature. Changes in the cerebrospinal fluid five hours after injection were fairly consistent. There was a slight rise in cerebrospinal fluid pressure, and usually a moderate rise in leukocytes. Erythrocytes were present in moderate numbers. Plasmin and streptodornase were present, and the amount of plasminogen fell, presumably as it was used up in conversion to plasmin by the action of streptokinase.

During the present investigation it appeared that the less abnormal the meninges were, the more severe would be the reaction to intrathecal enzymes. When the meninges were more diffusely diseased, as in the case of acute bacterial meningitis, the more benign would be the reaction. That the two groups of patients with tuberculous meningitis of different age groups could be separated on this basis is, however, hardly tenable.

Objective evaluation of beneficial effects in the treated patients was impossible in most instances. The 6 patients with tuberculous meningitis exhibited no striking improvements directly attributable to enzyme treatment. In this group, evidence for enzymatic lysis of spinal subarachnoid block was obtained in 3 instances. However, the block recurred in each case. In 2 of the 4 cases of acute purulent meningitis, a definite beneficial effect was demonstrated: in one the striking thinning of the thick purulent cerebrospinal fluid; in the other disappearance of neurologic abnormalities shortly after administration of enzymes. Eradication of *Ps. aeruginosa* in the last case presented was unequivocal.

The development in the cerebrospinal fluid of antibodies to streptokinase two to four weeks following intrathecal enzyme therapy was demonstrated in several patients. In the only instance in which this seemed to prevent the formation of an active fibrinolytic system (*Case 3*), the antibody could be overcome by employing large amounts of enzymes as well as serum as a source of plasminogen. The development of antibody can only be detected by actually measuring it in the cerebrospinal fluid. It was concluded that such mensuration was most important in carrying out protracted enzyme therapy.

Summary

With the hope of effecting dissolution of subarachnoid exudates, streptokinase and streptodornase were employed intrathecally as an adjunct to antibacterial agents in the treatment of a small series of patients with advanced tuberculous meningitis as well as patients with other types of bacterial meningitis. Particular attention was paid to assay of the enzyme systems in the cerebrospinal fluid at various intervals following intrathecal therapy.

A peculiar reactivity of the meningeal membrane was characterized by transient but severe toxic irritative reactions in the older patients with tuberculous meningitis and in subjects with normal cerebrospinal fluids, as opposed to minimal or complete lack of toxicity in the young children with tuberculous meningitis and in all of the patients with acute bacterial meningitis. Those patients who showed the toxic reaction failed to show active enzyme systems in the cerebrospinal fluid, presumably due to the inflammatory outpouring of inhibitory substances from the serum. In those patients not exhibiting toxicity, active enzyme systems were demonstrated in the cerebrospinal fluid for several hours following injection.

Dissolution of spinal subarachnoid block was accomplished in 2 patients, but only temporarily, due to causes which are discussed.

Although clinical results of treatment of the acute bacterial meningitides were encouraging, objective evaluation of the influence of streptokinase-streptodornase on tuberculous meningitis could not be made because of the small number of patients treated.

Sumario

Estudios del Uso Intratecal de la Estreptoquinasa-Estreptodornasa en las Meningitis Tuberculosa y Bacterianas de Otras Formas

Con la esperanza de conseguir la disolución de los exudados subaracnoideos, se emplearon intratecalmente la estreptoquinasa y la estreptodornasa como coadyuvantes de agentes antibacterianos en el tratamiento de la meningitis tuberculosa así como en enfermos con otras formas de meningitis bacterianas. A varios plazos consecutivamente a la terapéutica intratecal, prestóse atención particular a la valoración de los sistemas de encimas en el líquido céfalorraquídeo.

Una reactividad peculiar de la membrana menfngea se caracterizó por graves, pero pasajeras, reacciones irritantes tóxicas en los enfermos de edad que padecían de meningitis tuberculosa y en los sujetos que tenían líquido céfalorraquídeo normal, en contraposición a la toxicidad mínima o atoxicidad absoluta observada en los niños pequeños que padecían de meningitis tuberculosa y en todos los enfermos con meningitis bacteriana aguda. Los sujetos que revelaron la reacción tóxica no mostraron sistemas activos de encimas en el líquido céfalorraquídeo, presuntamente debido a la efusión inflamatoria de substancias inhibidoras del suero. En los enfermos que no manifestaron toxicidad, observáronse sistemas activos de encimas durante varias horas después de la inyección.

En dos enfermos, se logró, aunque sólo temporalmente, la disolución del bloqueo subaracnoideo raquídeo, debido a causas que se discuten.

Si bien fueron alentadores los resultados clínicos del tratamiento de las meningitis bacterianas agudas, no pudo llevarse a cabo la justipreciación objetiva del influjo de la estreptoquinasa-estreptodornasa sobre la meningitis tuberculosa debido al pequeño número de enfermos tratados.

Resume

Etude sur l'emploi de la streptokinase et de la streptodornase par voie sous-arachnoïdienne dans la méningite tuberculeuse et autres types de méningite bactérienne

Dans l'espoir de provoquer la dissolution des exudats sous-arachnoïdiens, la streptokinase et la streptodornase ont été administrées par voie sous-arachnoïdienne comme adjuvant aux agents antibactériens, aussi bien dans le traitement de la méningite tuberculeuse que dans les autres cas de méningite bactérienne. Une attention particulière a été accordée à la détermination des systèmes enzymatiques dans le liquide céphalo-rachidien, à intervalles variés, à la suite du traitement sous-arachnoïdien.

Une réactivité particulière des méninges était caractérisée par des réactions irritatives sévères chez les sujets âgés atteints de méningite tuberculeuse et chez l'individu ayant un liquide céphalo-rachidien normal, par contraste avec l'absence complète ou minime de toxicité observée chez le jeune enfant atteint de méningite tuberculeuse et chez tous les malades atteints de méningite bactérienne aiguë. Chez les malades qui présentaient ces réactions d'intolérance, les systèmes enzymatiques dans le liquide céphalo-rachidien étaient dépourvus d'activité, sans doute à cause de l'épanchement de substances inhibitrices plasmatiques d'origine inflammatoire. Chez ceux des malades qui ne présentaient pas de réactions d'intolérance, l'activité des systèmes enzymatiques a été démontrée dans le liquide céphalo-rachidien durant plusieurs heures après l'injection.

La dissolution du bloc sous-arachnoïdien rachidien a été réalisée chez deux patients, mais seulement temporairement, pour certaines raisons qui sont commentées.

Bien que les résultats cliniques du traitement des méningites bactériennes aiguës soient encourageants, une évaluation objective de l'influence de la streptokinase-streptodornase sur la méningite tuberculeuse n'a pu être effectuée en raison du petit nombre de malades traités.

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THE SURGICAL TREATMENT OF SPONTANEOUS IDIOPATHIC HEMOPNEUMOTHORAX[1,2]

A Review of the Published Experience with a Report of Thirteen Additional Cases

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INTRODUCTION

Spontaneous hemopneumothorax was known to Laennec, Littre, and others, according to Hopkins (51), but the early writings are obscure concerning the exact nature of the malady described. Boland (11), Rolleston (103), and Pitt (94) are credited with being the first to describe accurately and name the condition when they reported cases in 1900.

IDENTITY

The condition known as spontaneous hemopneumothorax is closely related to pneumothorax, to hemothorax, and to traumatic hemopneumothorax with respect to its origin, course, and treatment. Pneumothorax usually precedes spontaneous hemopneumothorax by a variable interval, but the hemorrhage and escape of air into the chest are sometimes synchronous. Nontraumatic hemothorax is a very rare entity but has been known to occur (8, 24, 57, 93). A case of spontaneous hemopneumothorax (9) has been recorded in which hemothorax occurred first and later pneumothorax took place. The role of the aspirating needle as a traumatic instrument must not be overlooked in the etiology of this form of pneumothorax. Conversely, the aspiration of air in pneumothorax may result in traumatic hemothorax which can be misinterpreted as spontaneous hemopneumothorax.

Often the relation of trauma to pneumothorax and hemopneumothorax is not clear. Undoubtedly cases which have been classed as spontaneous have had obscure trauma as the precipitating factor. Two cases will be mentioned in table 2 which, except for their traumatic background, would have been called spontaneous hemopneumothorax.

For the purposes of this work, all cases of spontaneous hemopneumothorax associated with tuberculosis (10, 51, 71) or other pulmonary disease, except cystic disease, have been eliminated from consideration. For example, in a case reported by Birch (10), the patient had a small tuberculous cavity in the lung which apparently was not involved in the spontaneous hemopneumothorax. This case might have been idiopathic but, because of the associated tuberculosis, it is not included here.

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INCIDENCE

Many conflicting statements are present in the literature (51, 81, 86, 102) with respect to the number of cases of spontaneous hemopneumothorax which have been reported. These incompatible figures are due to an inadequate examination of the literature and also, perhaps, to the different criteria used in classification. In a comprehensive review of the subject of spontaneous hemopneumothorax to 1937, Hopkins (51) collected 38 cases of this entity, excluding those in tuberculous patients. The present writers have found 12 cases reported in this period which were not among those collected by Hopkins. All of the known reported cases of idiopathic spontaneous hemopneumothorax until the present time are tabulated in table 1.

The statement that spontaneous hemopneumothorax is a rare condition occurs time and again in the literature. Hyde and Hyde (56) stated that it is not rare. Minor, in discussing a paper written by Wilson (129), said that it was not as rare as would be indicated by the published cases. Without entering into this controversy, it is believed by the present writers that hemorrhage into the pleura complicating spontaneous pneumothorax is a relatively rare complication of a rather common condition. Its incidence, reported by Hyde and Hyde (56) in the largest series (12 cases) yet published, was 4 per cent of their cases of spontaneous pneumothorax. Of the present writers' own cases of spontaneous pneumothorax, 8.3 per cent have been complicated by a considerable amount of bleeding. The total number of cases recorded in the literature to date has been 174. Most of the case reports have been single. Undoubtedly the incidence of spontaneous hemopneumothorax is much greater than would be indicated by the published cases.

The sex incidence is preponderantly in males. In all of the cases reported to date, only 6 have been in females. These were reported in 1937 by Hopkins (51), in 1945 by Waring (126), in 1949 by Dorset and Terry (31) and by Hansen (42), in 1950 by Keretsz (62), and in 1953 by Freund and Hicks (37). To this may be added one more case of spontaneous hemopneumothorax in a woman (*Case 7* of the present series). A case of spontaneous hemopneumothorax was reported in a woman by Liston and Lamberti (71). This woman, however, had pulmonary tuberculosis. The sex incidence of spontaneous hemopneumothorax seems to parallel roughly the sex incidence of spontaneous pneumothorax, as would be expected.

The age incidence of spontaneous hemopneumothorax approximately parallels that of spontaneous pneumothorax. However, there are much wider extremes in the occurrence of simple spontaneous pneumothorax. They vary all the way from birth to advanced old age, but most cases occur in the period from twenty to forty years.

Many writers have stated that most cases of spontaneous hemopneumothorax occur between the ages of twenty and forty. An interesting observation in the present survey of the literature and in the writers' own cases is that the age incidence in most cases of spontaneous hemopneumothorax is somewhat lower than is popularly supposed. The maximum incidence is between the ages of seventeen

TABLE 1

TOTAL PUBLISHED CASES OF SPONTANEOUS IDIOPATHIC HEMOPNEUMOTHORAX*

Year	Author	Number of Cases
1900	Boland (11)	1
	Rolleston (103)	1
	Pitt (94)	1
1910	Ness and Allen (87)	1
1913	Bushby (18)	1
1917	Williamson (128)	1
1922	Fischer (35)	1
1923	Kiaer (63)	1
1926	Bouchert and Beaupere (13)	1
1928	Doria (30)	1
	Hurxthal (54)	1
1930	Terry (121)	1
1931	Palmer and Taft (88)	2
	Milhorat (78)	1
	Housden and Pigott (52)	1
1932	Kjaergaard (67)	2
1933	Woll (130)	1
1934	Leggett *et al.* (70)	1
	Mayer *et al.* (70)†	1
	Bellon (7)	1
1935	Staffieri (117)	1
	Frey (38)	1
	Castex and Mazzei (20)	1
	Holden (49)	1
	Aguilar and Ferradas (1)	2
	Wilson (129)	1
	Catuogno (21)	2
	Rossell (106)	1
	Braco and Braco (15)	1
	Amberson‡	3
	Minor‡	1
1936	Centeno *et al.* (22)	1
	Korol (68)	1
	Vio (125)	1
	Poli (95)	1
	Jones and Gilbert (59)	1
	Munoz Moratorio (82)	2
	Troisier *et al.* (123)	1
1937	Hopkins (51)	3
	Hees (47)	1
	Cardenas (19)	1
	Rist (100)	1
1938	Euges (34)	1
	Louria (73)	4
	Maxwell (75)	1
	Perry (93)	1
	Salaris (108)	2
	Beaumont (6)	2
1939	Sloer (113)	1
	Tisi *et al.* (122)	1
1940	Davidson and Simpson (27)	1
	Rist and Worms (101)	1
	Quinlan (96)	1
	Hansen and Repetti (43)	1
	Remolar (99)	1
	Lorge (72)	1
	Jennings (58)	1
1941	Berliner (8)	4
	Rodriguez Pastor (102)	2
	Landron§	1
1942	Hartzell (45)	4
	Lea (69)	1
	Tannenbaum (120)	1
	Snively *et al.* (115)	1
1943	Staffieri (118)	1
	Liston and Lamberti (71)	1
	Raimondi and Boffi (97)	1
1945	Montoro (80)	1
	Payn and Lief (92)	1
	Waring (126)	2
	Sellors (111)	1
1946	Van der Meer (124)	1
	Harrington and Frelick (44)	1
	Helwig (48)	1
1947	McMyn (76)	2
	Stockler (119)	4
1948	Elrod and Murphy (33)	1
	Crimm (25)	1
	Orsi (90)	2
1949	Nario *et al.* (86)	2
	Dorsett and Terry (31)	1
	Nalls and Matthews (84)	3
	Solovny (116)	1
	Hansen (42)	4
1950	Granadeiro (40)	1
	Arst *et al.* (2)	2
	Deiss *et al.* (28)	2
	Keretsz (62)	1
	Cunningham (26)	2
	Deucher (29)	2
	Cosgriff (23)	1
1951	Beatty and Frelick (4)	1
	Hyde and Hyde (56)	12
	Moser (81)	4
	Meyers *et al.* (83)	1
	Seley and Neuhof (110)	1
	Josselevich *et al.* (60)	1
	Grimaldi (41)	1
1952	Fusia and Cook (39)	2
	Beatty and Frelick (5)	5
	Kastil (61)	2
	Eddinger and Reuben (32)	3
	Holloway *et al.* (50)	1
	Kieckens (64)	1
	Ross (104)	6
	Borrie (12)	2
1953	Ross *et al.* (105)	1
	Freund and Hicks (37)	1
	Huss (55)	1
	Present authors	13

* An additional case of spontaneous hemopneumothorax with recovery, not included in this table, was reported by Dr. Josef May in Medycyna, 1937, No. 15, pp. 536–538.

† Quoted by Beaumont.

‡ Discussion of paper by Wilson.

§ Quoted by Rodriguez Pastor.

and thirty-two. This may be significant in the consideration of the etiology of spontaneous hemopneumothorax, to be discussed hereafter. The average age incidence of spontaneous idiopathic hemopneumothorax for all the known reported cases is twenty-eight years.

Although the statement has been made (37) that 80 per cent of spontaneous hemopneumothoraces occur on the right side, review of the reported cases does not bear this out. In fact, there is almost no difference in incidence of the condition in the two sides, in considering all of the known cases.

The geographic incidence of spontaneous idiopathic hemopneumothorax, indicated by the number of cases reported, is as follows: North America, 98; Europe, 45; South America, 27; New Zealand, 4. These data do not permit any deductions concerning the actual distribution of the cases throughout the world. Data are lacking from many countries with vast populations but scant medical literature.

The racial incidence of spontaneous idiopathic hemopneumothorax, judging by the published cases, is almost totally in whites. Two cases have been reported in Negroes. One case occurred in a Chinese student. Does this indicate a possible predilection for pleural bleb formation on the part of whites? As in the geographic incidence, the racial incidence revealed by published cases is not trustworthy because of incomplete data. Hence, no definite conclusion can be drawn. One of the patients of the writers' series was a Negro.

ETIOLOGY

The etiology of spontaneous pneumothorax and hemopneumothorax has been the subject of much speculation (65, 66). Twenty years ago it was popularly supposed that nearly all spontaneous pneumothorax was of tuberculous origin. Not until the past decade has the role of subpleural blebs and bullae become appreciated in the production of spontaneous pneumothorax. The assumption that the rupture of such blebs and bullae is also the cause of hemopneumothorax may be correct in part. In hemopneumothorax the pneumothorax nearly always occurs first and the complicating hemorrhage occurs later or is recognized later. Rarely is evidence of massive hemorrhage noted when the patient is first seen by the physician. It appears that the original collapse of the lung might frequently result from the rupture of a subpleural bleb. In some instances in which the thorax has been opened at operation or at autopsy, no other lesion was found to account for the intrapleural bleeding (27, 40, 72).

The development of these subpleural vesicles is a controversial subject and has been discussed at length by Macklin and Macklin (74) and by Ornstein and Sercher (89). Kjaergaard (67) was the first to suggest that pneumothorax of the spontaneous variety was due to the rupture of a cyst. That this mechanism is a common one in the production of spontaneous pneumothorax has been amply confirmed by autopsy and operation. In fact, all of the present writers who operate upon patients for recurrent pneumothorax have observed that the pneumothorax was caused by the rupture of subpleural blebs. To be strictly accurate in the terminology, it would be better to designate the entity as spontaneous vesicular pneumothorax rather than spontaneous idiopathic pneumo-

thorax in all those cases proved to be due to the rupture of blebs, cysts, or bullae. Brock (14) has reported porous lungs as a cause of spontaneous pneumothorax. The present writers have had no experience with this condition.

Intrapleural bleeding in significant amounts, it is believed, is more likely to come from the chest wall or the mediastinum than from the collapsed lung. The systemic arterial pressure being greater than the pulmonary pressure, bleeding would proceed at a greater rate from the systemic vessels, if torn, than from pulmonary vessels. The elasticity of the lung promotes collapse, which aids in the stasis of visceral bleeding, whereas the vessels of the chest wall and mediastinum are more rigidly held and cannot contract to as great an extent as the pulmonary vessels. Pressure of air and blood in the closed thorax also tends to control lung bleeding by tamponade (46). The fact that torn adhesions are often found to be the source of bleeding from a vessel in the chest wall or mediastinum makes adhesions an important factor in the production of hemopneumothorax. First, a bleb ruptures and the elastic pull of the lung or the positive air pressure in the thorax causes a vascular adhesion to tear, resulting in intrapleural bleeding. The same mechanism might result in visceral bleeding if the adhesion failed to part but continued to resist contraction of the lung.

In 14 patients with fatal hemopneumothorax who were examined post mortem, Helwig and Schmidt (48) reported ruptured adhesions in 4 patients, ruptured blebs in 5, and no bleeding point in 5 patients. In each of these latter, it appeared that a bleeding point had been present until the patient became exsanguinated or until the blood pressure approached zero. It is reasonable to assume that this bleeding point was in a systemic vessel.

The writers' own experience, as well as that of other surgeons (2, 26, 83), has shown that massive bleeding in spontaneous hemopneumothorax usually comes from torn vascular adhesions. Although this source of the bleeding has long been suspected, surgical intervention is now revealing more cases to be due to this cause. It apparently has been shown at autopsy (48) that the source of bleeding in some instances has been from the lung. In one of the surgical cases of the present series, this appeared to be the only source of bleeding. The lacerated lung is admittedly a source of hemorrhage in spontaneous pneumothorax. A case of this kind was mentioned by Pardal and Mazzei (91) in which the rupture of a cyst caused a tear into a vascular region of the lung. It is believed, however, that cystic lungs, emphysematous lungs, and lungs containing subpleural vesicles are seldom vascular in the vicinity of the cystic change. In fact, avascularity may be the underlying condition from which the cysts result.

The age and sex incidence may point to a factor in the etiology of spontaneous pneumothorax and spontaneous hemopneumothorax. It may be significant that these conditions occur almost exclusively in males during the period of their greatest physical vigor and activity. At first glance it is difficult to reconcile the fact that so many cases have their onset while the patient is at complete rest, with activity as an etiologic factor. This suggests that the activity of the person might cause an increase of tension in the pulmonary bleb or bulla which, subsequently becoming attenuated, ruptures (resulting in the pneumothorax), and

is followed later by the tearing of a vessel and hemorrhage. Such a period of delay has been noted in traumatic hemopneumothorax between the time of injury and the development of the pleural bleeding.

Many reported cases of spontaneous idiopathic pneumothorax and hemopneumothorax may be erroneously classified as such. Trauma may have been the actual cause of the condition or the precipitating factor. For example, Euges (34) reported a case occurring as a result of exercising in a gymnasium. Jennings (58) reported a case which occurred in an air flight. The present writers have seen a case of spontaneous pneumothorax occur in a naval aviator.

Keretsz (62) reported the case of a young woman who fell to her knees in an attempt to catch a bus. She felt a sudden pain in the vicinity of the thoracic spine. One week later she developed a hemopneumothorax which was apparently spontaneous, but the history of the antecedent trauma certainly raises an interesting question of the possible effect of the trauma. Although trauma and disease in the etiology of this condition have been excluded from consideration for the purposes of this paper, it must be realized that these conditions cannot always be excluded in borderline cases. In fact, pulmonary disease may in some instances coexist and have no bearing on the hemopneumothorax. Such a case is the one reported by Birch (10).

CLINICAL COURSE

The onset of hemopneumothorax is almost always accompanied by pain of more or less severe nature. The pain may radiate to the abdomen and require differentiation from that of an acute surgical abdominal emergency (54, 78, 103). The present writers have seen this occur in lobar pneumonia, but none of the cases of hemopneumothorax in the present series have presented such radiation of pain. The pain may simulate the pain of coronary arterial disease (71, 113), perforated peptic ulcer (42, 104), or renal disease (25). Laparotomy has been considered in several cases of painful hemopneumothorax and has actually been performed (104) because of the error in diagnosis. The pain is often described as mild at the onset followed by a remission, and later a more severe episode of pain supervenes. This possibly represents the sequence of onset of the pneumothorax, collapse of the lung, and tearing of an adhesion with intrapleural hemorrhage. Signs and symptoms of intrapleural tension are frequently observed. There may be a shift of the mediastinum toward the uninvolved side. Shock is often noted when the hemorrhage has been great, the patient apprehensive, or the mediastinum and heart markedly displaced.

PROGNOSIS

The outlook for survival in spontaneous hemopneumothorax seems to be improving in recent years. Fatal cases are still being reported, however. Arst and associates (2), in 1948, stated that there was a mortality of 30 per cent in hemopneumothorax. Two patients in the present series came perilously near to death as a consequence of the hemopneumothorax. The loss of blood may be tremendous. As much as 10 liters of blood have been withdrawn from the pleural space

in a single case (128). The greatest measured blood loss in any of the present cases was 5,750 ml. The number of fatal cases of spontaneous idiopathic hemopneumothorax reported to date is 20, or approximately 12 per cent of all cases.

MORBIDITY

The over-all morbidity and loss of time from work is second only to the high mortality rate as an unfavorable sequela of spontaneous hemopneumothorax. The time usually required for expansion of the lung following removal of the blood is approximately two months, on the average. Waring (126) wrote of a patient who was clinically well 150 days after onset while he was roentgenographically well in 180 days.

Fibrothorax (figure 1) with incarceration of the lung often results from the defibrination of the intrapleural blood, in which case the lung cannot expand. Clotted hemothorax also causes the lung to become bound in a fibrous pleural scar and often results in immobilization of the diaphragm.

The fact that blood aspirated from the pleura in spontaneous hemopneumothorax usually does not clot has been responsible for the erroneous idea of many observers that blood in the pleura does not clot. Melick and Spooner (77), in their studies on experimental hemothorax, proved that blood in the pleura does clot. This has been confirmed clinically in the present series on the occasion of opening the chest in a case of recent hemopneumothorax. Sellors (111) has pointed out that, when dry taps of the chest are encountered in the presence of hemothorax, they indicate the hemothorax has clotted.

Studies on the blood in intrapleural hemorrhage have been going on for at least a century. The subject has recently been treated by Cosgriff (23) and Melick (77).

The experience of many investigators has shown that, soon after the onset of intrapleural hemorrhage, the cell content of blood aspirated from the pleura is approximately equal to that of the circulating blood. As time elapses after the onset of the hemorrhage, the blood in the pleura becomes progressively more dilute as a result of the serous effusion from the pleural surface in response to the irritating effect of the blood in the pleural sac.

An erythrocyte count on the hemorrhagic fluid is valuable in assessing the amount of recent intrapleural hemorrhage. If the cell count is high, it is indicative of recent or continuing hemorrhage. If the erythrocyte count is low, it indicates a large admixture of serous effusion with the likelihood that there has been little or no recent bleeding. When a diagnostic chest aspiration reveals blood which fails to clot, it is likely that the hemorrhage into the pleura has ceased or that bleeding is very slow. If, on the other hand, the blood clots, the assumption is that there is active bleeding. The blood in the pleural cavity becomes rapidly defibrinated and does not clot when aspirated in most instances of spontaneous hemothorax.

PRESENT SERIES OF 13 CASES OF SPONTANEOUS IDIOPATHIC HEMOPNEUMOTHORAX

The collective experience of the present writers in the treatment of spontaneous idiopathic hemopneumothorax consists of 13 patients, 8 of whom were

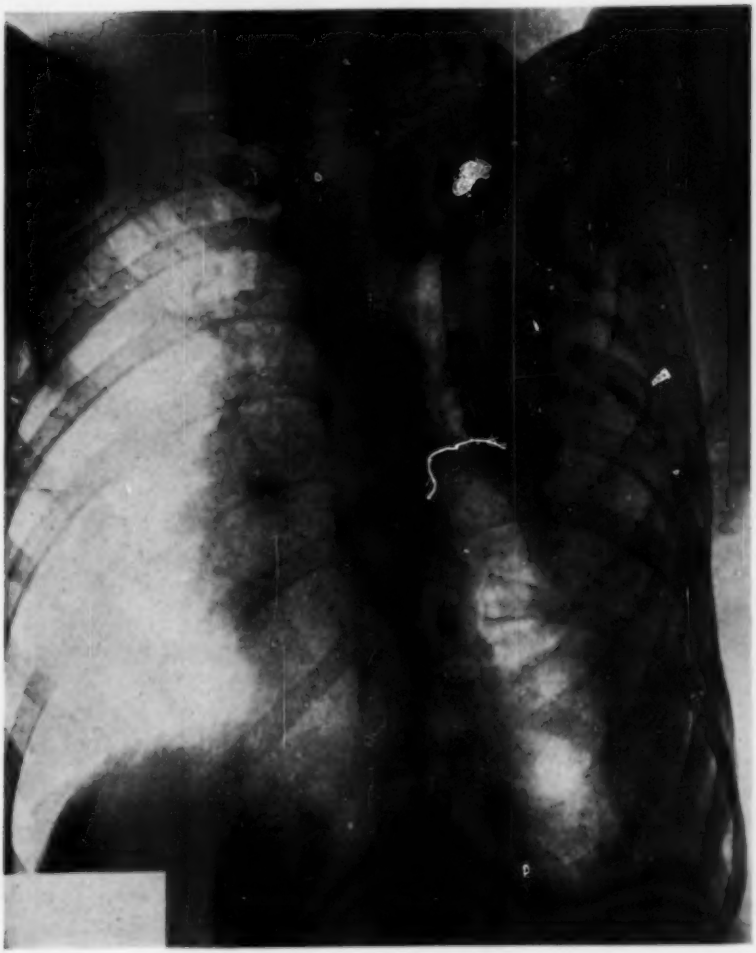

FIG. 1. Photograph of a roentgenogram of the chest reproduced from an article by C. S. Williamson in *The Medical Clinics of Chicago*, May, 1917, p. 1170. The photograph was made from a film exposed nine years after the episode of hemopneumothorax. This roentgenogram, made thirty-six years ago, illustrates pointedly what is now recognized as fibrothorax, a common sequela of hemopneumothorax. (*Reproduced with permission of W. B. Saunders Company*)

treated in the U. S. Naval Hospital, Oakland, and 5 of whom were treated by private physicians. The relevant data concerning these 13 cases are presented in table 2.[4] In an effort to avoid prolongation of the presentation, the individual

[4] Details of the individual case reports are available in the appendix to the reprint of this article.

TABLE 2

RESUMÉ OF THE PRESENT SERIES OF THIRTEEN PRESENTLY UNREPORTED CASES

Case	Race	Sex	Age	Symptoms	Blood Loss	Aspiration	Treatment	Operation
					c.c.			
1	W	M	27	Pain, chest	4,955	Yes	Transfusions	Decortication
2	W	M	20	Pain, chest	5,070	Yes	Transfusions	Primary thoracotomy; ruptured cyst and torn adhesion
3	W	M	30	Pain, chest	3,400	Yes	Transfusions	Decortication
4	W	M	23	Pain, chest; dyspnea	?	Yes	Transfusions	Primary thoracotomy; ruptured bleb and torn adhesion to subclavian vein
5	W	M	19	Pain, chest	2,920	Yes	Transfusions	None
6	W	M	19	Pain, chest	4,150	Yes	Transfusions	Decortication*
7	W	F	21	Pain, chest; dyspnea; shock	?	Yes	Transfusions	Primary thoracotomy; bleeding point on chest wall
8	W	M	27	Pain, chest; tight sensation	5,750	Yes	Thoracoscopy, transfusions	Primary thoracotomy
9	W	M	51	Pain, chest	1,450	Yes	Aspiration and bed rest	None
10	W	M	27	Pain and pressure, chest	?	Yes	Transfusions	Thoracotomy; ruptured bleeding bleb
11	W	M	31	Pain, chest	3,500	Yes	Transfusions	Primary thoracotomy; ruptured bleb; torn adhesion to subclavian vein
12	N	M	33	Pain, chest; dyspnea	1,540	Yes	Transfusion	None
13	W	M	19	Pain, chest; dyspnea, syncope	1,820	Yes	Aspiration and bed rest	None

* *See* figure 2 in text.

case records are not presented in the text but are referred to by the case numbers in the table.

Although this paper deals primarily with spontaneous hemopneumothorax, 2 cases of traumatic origin will be briefly mentioned in which the intrathoracic hemorrhage was delayed and did not occur immediately subsequent to the original trauma but was severe enough to necessitate surgical intervention.

The first of these patients had been crushed between two Greyhound busses, sustaining fractures of six ribs on one side and seven on the opposite side, together with fractures of both clavicles. He had a bilateral partial pneumothorax. On the twelfth day of his hospitalization he began to show signs of hemorrhage into the left pleura. Thoracotomy was resorted to and two bleeding intercostal vessels were ligated, proximal and distal to their points of bleeding.

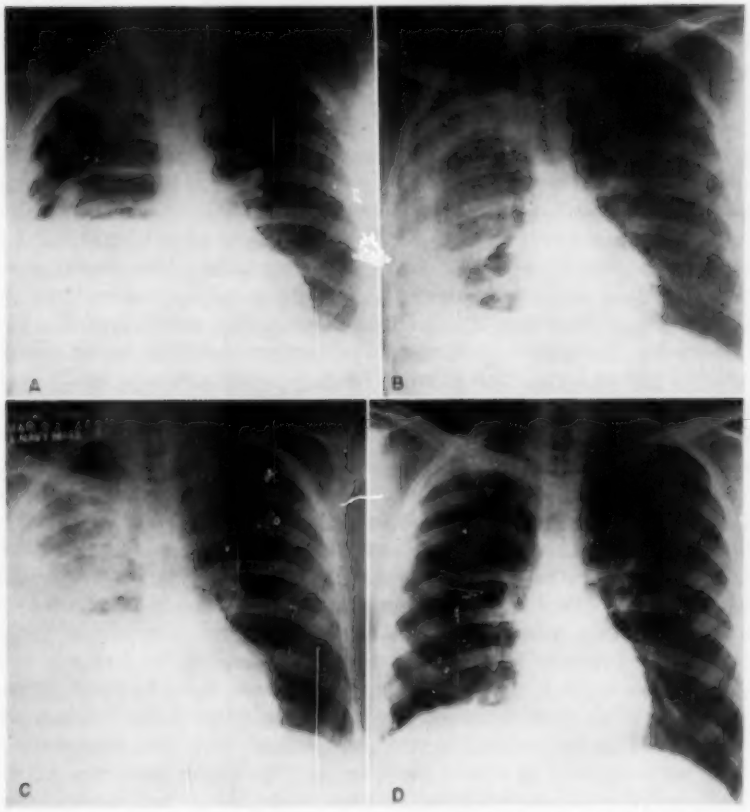

FIG. 2. Reproductions of roentgenograms of the chest (*Case 6*). *A*. Right spontaneous hemopneumothorax with fluid level to third rib anteriorly. This film was exposed shortly after admission of the patient to the hospital. *B*. Chest film obtained one month later showing unexpanded lung and fluid level above the first rib anteriorly. *C*. Chest film taken seven weeks after admission showing partly expanded lung with fluid loculations. *D*. Chest film obtained one month later than that shown in *C*, and following decortication. The lung is completely expanded.

The second case was that of a young man who had been injured in an automobile accident. He had received fractures of two ribs and the left femur. The intrapleural hemorrhage in his case did not begin until fourteen days after arrival in the hospital. The bleeding was massive. The blood was aspirated, and replacement was made by transfusion. Thoracotomy and decortication of the lung were performed in this case two weeks later.

These examples illustrate what would ordinarily be considered spontaneous hemopneumothorax except for the fact that severe trauma preceded the bleeding by approximately two weeks in each instance. In this connection, it is believed that in many cases of spontaneous pneumothorax, with or without hemorrhage,

there might have been trauma or exertion of recent date which was the inciting factor, although the clinical manifestations did not appear until after the trauma or exertion had been forgotten or was considered irrelevant.

TREATMENT

Many recommendations have been made for the treatment of spontaneous idiopathic hemopneumothorax and many conflicting ideas have been expressed. Some have believed that the condition will run a self-limited course and requires no active treatment (47), such as aspiration of the chest. The statement (92) has been made that "the disease usually runs a favorable course resulting in absorption of blood and air, treatment should therefore be conservative." It is difficult to reconcile a view of this kind with statistics which bespeak a 12 per cent mortality. Certainly a conclusion of this nature should be drawn only after experience with at least several cases. This lazy, nonintervention policy is to be condemned. Barrett (3) was the first to take exception to this neglect of the patient which passed as a form of treatment. He believed in active treatment of hemothorax.

Aspiration: Wilson (129) advised complete removal of bloody effusion to avoid clot formation and risk of pleural symphysis. He advocated delay in removal until evidence was present that the intrapleural hemorrhage had ceased. Fortunately for their patients, most of the physicians who have treated this affliction have aspirated the blood from their chests. The removal of blood is the surest prophylaxis against incarcerated lung and fibrothorax, which is a disabling sequela of hemopneumothorax. In spontaneous pneumothorax it is wise to permit the wound in the lung parenchyma to seal before trying to force expansion by aspiration of air from the chest. A short period of two to forty-eight hours is permitted to allow the perforation in the lung to seal. After that time continuous suction can be applied to expand the lung quickly. This suction treatment has been successful in a high percentage of cases. In the case of hemopneumothorax, the blood should be withdrawn early to prevent serious compromise of respiratory function as a result of the hemopneumothorax.

Although the present writers believe that the physician owes it to the patient to see that his pleura is kept as free of blood as possible, there is no unanimity of opinion on this subject. Some writers (46, 58) who believe in aspiration of the blood from the pleura recommend the introduction of air for the purpose of tamponade to prevent further hemorrhage as a result of decreasing the intrapleural pressure. Louria was of the opinion that evacuation of blood might dispose to further hemorrhage and bronchopleural fistula. The advantage derived from the aspiration of blood probably outweighs any danger from this procedure.

Thoracoscopy: Thoracoscopy for verification of the diagnosis and for treatment has been used and/or advocated by several writers (10, 19, 42). One of the patients in the present series (*Case 8*) was subjected to thoracoscopy. It proved to be a wasted effort, as the patient subsequently required open operation. The use of thoracoscopy in this condition is certainly limited.

Thoracostomy: Thoracostomy and tube drainage have been advocated by

Brunner (17), Hughes, Kraeft, and Lowry (53), and Brown and Boyd (16). Two of the patients in the present report (*Cases* 4 and 8) so treated were later treated by thoracotomy. At this point a warning is in order against the use of suction in the early evacuation of the blood in the chest, for severe hemorrhage may result. If tube drainage is employed, a simple underwater seal should be used. If bleeding is severe and continuous, thoracostomy may be only delaying the indicated thoracotomy.

Use of fibrinolytic enzymes: The use of streptokinase and streptodornase in hemothorax has been followed by brilliant results in the hands of some observers (79, 98, 112). The experience of the present writers with these enzymes, although limited, has been disappointing. This is mentioned only to show that uniformly good results are not obtained by all users of these enzymes. In spite of the poor results, the present writers believe that a trial of these fibrinolytic enzymes might possibly obviate decortication if used at the proper time, if the thorax has been as thoroughly evacuated of blood as possible, and in the absence of bronchopleural fistula.

Treatment to prevent recurrence of pneumothorax: The use of poudrage, instillation of silver nitrate, and other measures to cause pleural symphysis has been mentioned (42), but is probably unnecessary, as the recurrence of hemopneumothorax is extremely rare. The irritant effect of the blood in the pleura seems to be enough to cause adhesions which protect against recurrence. In fact, Rothkopf (107) advocated the use of artificial hemothorax as a cure for recurring spontaneous pneumothorax. The only case on record of recurrence of pneumothorax following spontaneous hemopneumothorax which has come to our attention was that reported by Rist (101). The patient had a recurrence of pneumothorax on the ipsolateral side two years following a hemopneumothorax. A case in the present series (*Case 13*) is the second known instance of pneumothorax to recur following spontaneous hemopneumothorax.

Primary thoracotomy: Primary thoracotomy in the treatment of spontaneous idiopathic hemopneumothorax, although rarely employed, is a rational procedure and in certain instances is mandatory as a life-saving measure. In the literature to date, this step has been taken in 7 instances (12, 29, 39, 50, 83, 104, 105) with satisfactory results. It is extremely doubtful if any of these patients would have survived except for the timely surgical intervention. Six of the patients in the present series were successfully treated by primary thoracotomy. Three of these were operated upon in extremis and made dramatic recoveries.

Primary thoracotomy is also to be considered as a means of decreasing morbidity and, as such, it has a distinct value. Three of the patients (*Cases* 1, 3, and 6) in the present series who had thoracotomy for decortication should have had primary thoracotomy rather than multiple aspirations and decortication. These involved massive hemorrhage and were of very grave nature. In retrospect it can be seen where primary thoracotomy to arrest the bleeding and expand the lung would have resulted in the saving of many man-days for these patients. One who developed a psychosis (*Case* 6) would probably have been spared this complication if the radical procedure had been carried out early in the illness.

From our experience with primary thoracotomy and decortication procedures in the present series, it is believed that the indications for surgical intervention to arrest hemorrhage are: the presence of initial severe hemopneumothorax, or continued hemorrhage as indicated by refilling of the pleural space after evacuation, severe shock, and deteriorating condition of the patient.

Blood transfusions, oxygen inhalation, and other supportive measures are of extreme importance. The aspiration of air and neutralization of air pressure, if tension pneumothorax is present, are required.

Eleven years ago, Hartzel (45) stated "Open operation in an attempt to find the bleeding point may then be considered if a hardy surgeon is available. There is no report of an attempted operative treatment of spontaneous hemopneumothorax among the cases which we have compiled." Of the 11 cases in the literature on which there are data concerning time of death, 5 patients with spontaneous hemopneumothorax died within twenty-four hours of admission (31). Primary surgical intervention is the only sure means of avoiding these early deaths. Hartzell's look into the future has been fulfilled in the last three years, for it is during this time that primary thoracotomy for severe and progressive spontaneous hemopneumothorax has come to be performed.

Decortication: In late cases of hemopneumothorax in which the lung has failed to expand, due to rapid defibrination of the pleural blood or to neglect on the part of the physician or surgeon to remove the blood from the pleura or to do so inadequately, surgical intervention becomes necessary for the purpose of removing the fibrous envelope which incarcerates the lung. In addition, the "peel" on the parietal pleura and diaphragm may require removal in order that the chest may expand normally with inspiration. The subject of decortication, its technique and indications, has been well covered in connection with traumatic hemothorax (85, 109, 114, 127). The same principles apply to the treatment of fibrothorax resulting from spontaneous hemopneumothorax.

At least three writers (33, 84, 110) have claimed priority in the use of this procedure in spontaneous hemopneumothorax. One of them delayed reporting his case for nearly fourteen years and did so only after two other surgeons had published their use of the operation in spontaneous hemopneumothorax.

Fourteen patients with spontaneous hemopneumothorax have been treated by decortication to date, as indicated by a survey of the reported cases. The present writers have had occasion to resort to this operation and have attained gratifying results in 3 cases of the present series.

SUMMARY

The incidence of spontaneous idiopathic hemopneumothorax, as revealed by analysis of all the known cases, is almost exclusively in white males between the ages of seventeen and thirty-two. Only six cases of this clinical entity have been reported previously in women. A seventh case has been added.

Further autopsy and clinical evidence has been presented to show that the origin of spontaneous idiopathic pneumothorax is usually from the perforation

of a vesicle, while the source of complicating severe hemorrhage is variable but most often is the result of a torn pleural adhesion containing blood vessels.

A wide variety of symptoms have been described in the literature, but the patients of the present series had only chest pain, dyspnea, and shock.

The mortality rate of spontaneous idiopathic hemopneumothorax appears to be improving, possibly due to prompt and improved handling of these patients. The mortality rate for all cases reported to date is approximately 12 per cent.

The morbidity is great in spontaneous hemopneumothorax due to the formation of fibrothorax and its attendant interference with pulmonary function.

The state of the blood in the pleura has been discussed briefly and its clinical significance interpreted.

In treatment, emphasis has been placed on primary thoracotomy as a life-preserving operation as well as a prophylaxis against morbidity. Decortication for late sequelae of spontaneous hemopneumothorax also has been discussed.

SUMARIO

El Tratamiento Quirúrgico del Hemoneumotórax Idiopático Espontáneo. Repaso de las Observaciones Publicadas con la Presentación de Otros Trece Casos

Según revela el análisis de todos los casos conocidos, la incidencia del hemoneumotórax idiopático espontáneo es casi exclusivamente en varones blancos de 17 a 32 años de edad. Sólo 6 casos de esta entidad clínica habían sido comunicados en mujeres, a los cuales se agrega ahora el séptimo.

Se ofrecen nuevos datos autópsicos y clínicos para demostrar que el neumotórax idiopatico espontáneo suele tener su origen en la perforación de una vesícula, en tanto que varía la causa de la hemorragia grave que lo complica, pero siendo más a menudo consecuencia del desgarro de una adherencia pleural que contiene vasos sanguíneos.

En la literatura se ha descrito una inmensa variedad de síntomas, pero en la serie actual los enfermos no tenían más que dolor torácico, disnea y choque.

La mortalidad del hemoneumotórax idiopático espontáneo parece ir mejorando, posiblemente debido a la asistencia rápida y mejor de estos sujetos. El coeficiente de mortalidad para todos los casos comunicados hasta ahora es aproximadamente de 12 por ciento.

La morbidad es considerable en el hemoneumotórax espontáneo debido a la formación de fibrotórax con la consiguiente obstrucción de la función pulmonar.

Discútese brevemente el estado de la sangre en la pleura, interpretándose su importancia clínica.

En el tratamiento, se ha recalcado la toracotomía primaria como operación salvavida e igualmente como profilaxis contra la morbidad. También se ha discutido la decorticación para las secuelas tardías del hemoneumotórax espontáneo.

RESUME

Le traitement chirurgical de l'hémopneumothorax idiopathique spontané. Un compte rendu des observations publiées et de treize cas additionnels

L'incidence de l'hémopneumothorax idiopathique spontané, révélée par l'analyse de tous les cas connus, est presque exclusivement observée chez les hommes de 17 à 32. Chez la femme, six cas seulement ont été antérieurement rapportés. Un septième cas a été ajouté.

Les auteurs présentent d'autres preuves cliniques et des résultats d'autopsie, qui démontrent que l'origine du pneumothorax idiopathique spontané est une perforation alvéolaire, tandis que la source de l'hémorragie sévère qui la complique est variable; elle est le plus souvent imputable à la déchirure d'une adhérence pleurale vascularisée.

Une grande variété de symptômes ont été décrits dans la littérature, mais les malades de la série actuelle n'avaient que des douleurs thoraciques, de la dyspnée et un état de choc.

Le taux de mortalité de l'hémopneumothorax idiopathique spontané semble s'améliorer, probablement à cause des soins plus prompts et meilleurs donnés à ces malades. Le taux de mortalité pour la totalité des cas rapportés est approximativement 12 pour 100.

La morbidité de l'hémopneumothorax spontané est élevée en raison de la formation d'un fibrothorax et du trouble de la fonction pulmonaire qu'il entraîne.

L'état du sang dans la plèvre est brièvement commenté et son importance clinique est interprétée.

En ce qui concerne le traitement, l'accent est mis sur la thoracotomie initiale comme l'opération qui préserve la vie du patient et prévient les séquelles morbides. La décortication contre les séquelles tardives de l'hémopneumothorax spontané ont été également discutées.

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THE RELATIONSHIP OF NONSPECIFIC AND SPECIFIC FACTORS IN THE TUBERCULIN REACTION[1, 2]

A Review

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INTRODUCTION

While some aspects of the mechanism of delayed hypersensitivity reactions, typified by the tuberculin test, have been made clearer in recent years, many features still remain obscure. Two observations stand out prominently, not only because of their own intrinsic significance, but also because of the lead that they have given to later research. The first was the finding by Rich and Lewis (1) that tuberculin is toxic to cells of hypersensitive animals when applied to them in tissue culture. The second, and more recent, was the observation by Landsteiner and Chase (2) and by Chase (3) that tuberculin and contact hypersensitivity can be transferred passively by inocula of cells of reticulo-endothelial origin and notably by lymphoid cells.

Studies in the present laboratory and in the literature on the influence of various specific and nonspecific factors on the tuberculin reaction are reviewed in this report in an attempt to discern a pattern of the events which lead to a combination of antigen and antibody and, hence, in turn usher in the inflammatory response.

The character and intensity of the tuberculin reaction can be influenced experimentally by various modifications of technique. For instance, it is possible to alter the availability or persistence of the antigen at the site of inoculation; the source and availability of the antibody; and the ability of the tissues to respond with an inflammatory response to the local combination of antigen and antibody.

PART I. THE AVAILABILITY AND PERSISTENCE OF THE ANTIGEN, TUBERCULIN, AT THE SITE OF INOCULATION

The persistence of the tuberculin at the inoculation site in the skin can be influenced considerably by agents which modify the local cutaneous circulation and the permeability of the capillaries and venules in the dermis. The changes which such agents bring about in the character of the reaction will be discussed as follows:

A decrease in the local persistence of tuberculin due to an enhancement of its rate of escape

An increase in the local persistence brought about by the use of slowly absorbing vehicles for the tuberculin or by induced local vascular inactivity

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[2] This study was aided in part by a grant from the British Medical Research Council.

The alterations in the rate and degree of local fixation of the tuberculin in the sensitized tissues

Decreased local persistence of tuberculin: When substances of large molecular size are injected intradermally, they escape mainly by way of local lymphatics. The rate at which this takes place depends largely on the speed of lymph formation and this, in turn, is related both to the activity of the blood circulation in the vicinity of the site and to the permeability of the vascular endothelium. Any increase in blood flow and capillary transudation thus leads, first, to the dilution of any soluble material injected and, later, to its removal from the region. It is scarcely surprising, therefore, that most nonspecific agents which act locally as lymphagogues should be capable of reducing, or even of inhibiting, an intracutaneous (Mantoux) reaction.

In various clinical conditions, too, such as pregnancy, the premenstrual period, some febrile and infectious disorders, hunger cachexia, and exposure to bright sunlight or other irritants, the Mantoux test is often partially or wholly inhibited. This inhibition has been attributed to various causes, such as immunologic anergy or to the inability of the tissues to produce an inflammatory response. In all of these conditions, however, the rate of disappearance of a standard intradermal saline bleb, as measured by the Aldrich-McLure test, is raised (4, 5*a*, *b*), and it may well be that the diminution of the Mantoux test is due to this augmented lymphatic drainage.

The effect of an erythema dose of ultraviolet irradiation, from a mercury vapor lamp or sunlight, on the Mantoux test has also been examined. Some workers have noted that the subsequent tuberculin reactions were more pronounced as long as no irradiation erythema had developed but, should this have appeared, the intensity of the reaction was minimized (6, 7*a*, 7*b*, 8). Aldrich-McLure tests have shown that the rate of disappearance of a saline bleb in the skin is enhanced after such irradiation (9), and the same accelerated dispersal of the injected tuberculin under these conditions might well account for the diminution of the Mantoux tests.

Effect of local histamine on the Mantoux test: When a wheal is produced, either by histamine or by an immediate type of hypersensitivity reaction, at the same time that some indicator material such as a dye, hemoglobin, or even erythrocytes is injected, the rate of escape of the latter from the site is much accelerated (10, 11). This enhancing effect of wheals on the rate of absorption of substances from the skin has been applied to a comparative study of the influence of certain nonspecific agents on the Mantoux test (12). The injection of 0.1 to 0.2 ml. of 1:1,000 histamine diphosphate into a Mantoux test site immediately after the inoculation of the tuberculin led to the complete suppression of reactions which, at control sites in the same subjects, were vigorous and sometimes necrotic.

From time to time, the present writer has observed that modified tuberculin reactions, presenting unexpectedly large areas of erythema, are produced when histamine whealing fails to produce complete inhibition of Mantoux tests which at control sites show vigorous reactions. These mitigated responses showed a negligible induration as compared with their controls and were far weaker in

intensity (12). It would seem that, although the exudation of the wheal had caused dilution and spreading of the tuberculin, it was insufficient to produce complete absorption. A larger area of lower concentration tuberculin had thus been left in the tissues. In tests on the effect of whealing on intradermally injected dyes, similar enlarged areas of faint coloration were occasionally seen.

In the course of this work, histamine wheals were produced at test sites before and at various intervals after the inoculation of tuberculin. Larger reactions, of lesser intensity than the controls, appeared at Mantoux tests made on sites where wheals had been produced one hour before the injection of the tuberculin. If the wheal was produced after the injection of the tuberculin, even though the delay might be only ten minutes, the inhibition was much less complete. The longer the interval between the tuberculin inoculation and the whealing, the less the inhibition so that, when the two injections were separated by thirty to forty minutes, the reactions at the experimental sites differed little from those at the control sites. These findings suggest that the tuberculin had reacted and become fixed in the tissues within a few minutes of its introduction.

In the light of such observations, it seems essential, when assessing the inhibitory powers of certain drugs on the Mantoux test, to take their nonspecific whealing properties into consideration.

For example, it has been stated that the injection of local anesthetic or antihistaminic drugs together with tuberculin inhibited the reaction; an effect which was ascribed to the anti-allergic or anti-inflammatory action of these substances (13). Both types of substance, however, are whealing agents in the concentrations which were found to be inhibitory, and it seems that, in these experiments, their action was due to their enhancing the rate of escape of the tuberculin from the site. The report that the local injection of cortisone together with tuberculin results in depression of the reaction (14) should also be viewed with the same possibility in mind, since the cortisone itself was found to evoke local erythema.

Effect of hyaluronidase on the Mantoux test: The superinjection of 0.10 ml. of a solution of hyaluronidase (Rondase®) containing concentrations of the enzyme which ranged from 1.0 mg. to 0.001 mg. per ml. led to an enlargement of the area of the reaction which was correlated with the amount of enzyme used; with the weakest concentration, the spread was little more than that produced by saline alone. The increase in the area of the reaction, however, was accompanied by some decrease of its intensity in the central area, although to a far less extent than that noted with histamine. The difference between the effects of the superinjection of these two substances is probably explicable on the assumption that, while both promoted the spread of the tuberculin, the histamine wheal had the additional effects of diluting the tuberculin and of accelerating the escape of much of it from the area as well. The failure of hyaluronidase, which had been applied to the surface of the skin, to influence the size of a tuberculin patch reaction was probably due to the method of testing (15).

Increased local persistence of tuberculin—"depot" tuberculin: The incorporation of tuberculin into a slowly absorbed vehicle, such as liquid paraffin or lanolin, has been used to prolong the local retention of tuberculin with the following

results (16*a*, *b*, 17, 18). Skin reactions to this "depot" tuberculin appear at the same time as parallel Mantoux reactions, but they are stronger and persist for several months.

In rabbits injected with "depot" tuberculin in the skin and infected one week later with tubercle bacilli, strong positive reactions developed at previously negative "depot" test sites sixteen days after the infection; at this time Mantoux tests were also positive.

In two humans, one of whom subsequently developed respiratory tuberculosis, previously negative "depot" test sites became positive after intervals of two and one-half and six months and persisted for approximately two years.

These observations resemble the "revivescence" reactions which appeared at previously negative Mantoux test sites in association with the development of active infection weeks or months after the test was made (19). The explanation for this is not known; it seems that the tuberculin must have persisted locally, although whether or not it had become fixed to the tissues is uncertain.

Effect of vascular stasis on the Mantoux and patch tests: Increased local persistence of tuberculin can also be effected by the retardation of lymphatic absorption from the dermis, either as a result of venous stasis or of a lesser arteriolar inflow of blood into the site. Investigations on the influence of venous stasis on the Mantoux test (4) have shown that larger reactions are produced on the arm below a vasoconstrictor band than above it. Reactions on paretic limbs with vascular stasis are also larger than those on normal limbs with an active circulation. It has also been recorded that treatment of the skin with carbon dioxide snow increased the rate of absorption from the skin, and that tuberculin tests carried out in the treated area were inhibited (5). Compression of the vessels around such areas, however, resulted in increased tuberculin reactions. The inhibition in the carbon-dioxide–treated skin could, therefore, not be attributed to loss of reactivity of edematous skin.

The present writer has found that, in tuberculin patch tests carried out on skin into which 0.05 to 0.10 ml. of epinephrine hydrochloride (1:1,000) had been injected, the reactions were much enhanced and showed a wider distribution of papules. On occasion, positive reactions developed when tests at control sites were negative (12). It seems likely that the epinephrine-produced vasoconstriction had decreased both the arteriolar inflow and the lymphatic absorption at the site. Such changes would lead to an increase in the local retention and concentration of the tuberculin penetrating the skin and thus to an enhanced reaction.

The effects of both histamine and epinephrine on the absorption of substances from the skin can be observed readily by using fluorescein as an indicator (12).

> Three intradermal injections of 0.05 ml. of sodium fluorescein (5 per cent) were superinjected with 0.05 ml. of histamine diphosphate (1:1,000), epinephrine hydrochloride (1:1,000), and normal saline, respectively. Subsequent examination under ultraviolet irradiation through a Wood's filter showed that at the histamine site most of the dye was absorbed by the end of one hour. After six hours, the dye had disappeared completely from both the histamine and saline control sites. At the

epinephrine-treated site, on the other hand, it had persisted much longer: after fourteen hours it showed as a vivid homogeneous fluorescent patch which slowly increased in area and was still easily recognizable at twenty hours.

These results clearly indicated the complementary effects of histamine and epinephrine in promoting respectively the disappearance or retention of intradermally injected substances.

Local "fixation" of tuberculin in the tissues: The rate and degree of "fixation" of the active constituent of tuberculin to sensitized structures has an important bearing on the mechanism of the tuberculin test. Even in unsensitized subjects, however, considerable local "fixation" occurs, as is shown by the well-known "revivescence" phenomenon. Of this nonspecific "fixation," few intimate details are yet known, although the ready adsorption of tuberculoproteins to glass surfaces and to the membranes of erythrocytes is amply attested (20*a*).

The rate of "fixation" in sensitized subjects may be estimated by the superinjection, at timed intervals, of various agents into Mantoux tests so that the area and nature of the reactions are altered (20*b*). The times arrived at for the onset and completion of fixation in the skin can be only approximate, since they have been deduced from modifications of a widely different character. In humans, the present writer has employed the following methods:

First, the degree of hypersensitivity of the subject was determined by prior testing with Old Tuberculin (OT). It was found that the most suitable concentration for these studies was that capable of exciting a moderately vigorous reaction. With weaker concentrations, dispersal of the tuberculin by the superinjected substances was associated with decreased reactions. Too strong concentrations, on the other hand, were undesirable as multiple tests were carried out simultaneously in each subject. A number of intradermal injections of 0.05 or 0.10 ml. of the appropriate concentration of OT (commonly 1:10,000 or 1:1,000) were given in the back, followed by the superinjection of 0.10 or 0.20 ml. of (*a*) normal saline, (*b*) histamine, or (*c*) hyaluronidase after the desired intervals. The nature of the reaction was best studied forty-eight hours later, whereas the area of reaction was most clearly defined five to seven days after the testing.

Superinjection of saline into the Mantoux test: This spread the tuberculin in the skin in a mechanical fashion against the resistance of the tissues. An increased area of reaction was produced if the saline was superinjected up to one to two hours after the tuberculin. This would appear to be the time needed for the complete fixation of a moderate dose of tuberculin, as tested by this method. The nature of the modified reaction was not altered, showing central induration and papulation surrounded by a zone of erythema and infiltration. The superinjection of saline increased the area of Mantoux tests in guinea pigs if given up to two and one-half hours after the test, according to Miles (personal communication).

Superinjection of hyaluronidase: The concentration of hyaluronidase employed for this purpose was 1 mg. per ml. of normal saline. The superinjection of the hyaluronidase into the Mantoux tests immediately after the introduction of the tuberculin altered both the nature and the area of the reaction. Instead of central induration and papulation, the modified reactions were increased in area and showed an even intensity of induration throughout, suggesting that the tuberculin had been

spread evenly throughout the area, owing to the decreased resistance of the tissue produced by the hyaluronidase.

As the interval between the two injections increased, so the reactions showed more and more intense central induration and papulation and less and less increase in the area of spread. An interval of ten to twenty minutes between the two injections was sufficient for the development of more marked central induration, and definite papules appeared at tests superinjected after a delay of thirty minutes. By the time one to one and one-half hours had passed between the two injections, there was often little difference as compared with control tests. When high concentrations of tuberculin were employed or when the subject was unduly sensitive, the superinjection of hyaluronidase spread the area of reaction, even after a delay of four to five hours. It seems, however, that sufficient tuberculin had become fixed within the first ten minutes to determine the appearance of the more intense central area of reaction.

Superinjection of histamine diphosphate: As described earlier, Mantoux tests are inhibited by the superinjection of histamine. The inhibition was far less if the histamine was superinjected after a delay as short as ten minutes. Tests superinjected after thirty to forty minutes were not markedly affected.

These results, like those found with hyaluronidase superinjection, suggest that tuberculin "fixation" commences early, within the first minutes after its injection. The nature and firmness of the "fixation" are more difficult to assess. Dispersal of the test substances in the tissues, making them inaccessible to the superinjected reagents, may play a part in the modified reactions produced. With histamine superinjected, for example, it was found that the larger the wheal produced, the greater the inhibition, suggesting that a more forceful dilution and "flushing away" of the tuberculin had occurred. When histamine was injected into deposits of intradermally injected dye, successive tests in the same subject showed varying, although decreased, amounts of residual dye, indicating that its absorption was not always equally enhanced. These findings must necessarily influence any deductions made in relation to the "fixation" of tuberculin. Consistently similar results were obtained, however, in large numbers of Mantoux tests into which the agents discussed were superinjected.

Some observations which may throw some light on the development of tuberculin reactions were made by Gernez and Marchandise (4). They found that the Mantoux test in humans was inhibited if a sharp bout of artificial pyrexia was elicited within five to seven hours after the tuberculin injection. It seems possible that the enhanced absorption from the skin, provoked by the persistent vasodilatation due to the pyrexia (4), may have decreased not only the local concentration of tuberculin, but may also have decreased the local persistence of inflammatory products which had been liberated in the early hours of the tuberculin reaction. On the other hand, the agents described above act for a short period only, and this may account for the differences observed.

Immediate type of reactions in tuberculin hypersensitivity: The relationship of immediate and delayed types of hypersensitivity is a challenging problem and may throw some light on the nature of the early "fixation" of tuberculin. The circumstances in which the subject is exposed to the antigenic material influences the production of the hypersensitive state. In the allergic states of infection and contact sensitization, both types of hypersensitivity appear.

Tuberculoprotein, like other proteins, produces an immediate type of hypersensitivity when injected by itself, and immediate types of reactions are elicited on subsequent testing. It can, however, elicit delayed reactions in subjects appropriately sensitized, as by active infection, and under these conditions it resembles a "partial" antigen since it does not by itself produce the delayed hypersensitive state. It is of considerable interest that proteins, which by themselves produce anaphylactic sensitization, have been reported to produce the delayed type of hypersensitivity if injected into tuberculous foci (21), and this applies also to tuberculoprotein and other proteins if they are injected together with purified tubercle wax (22). Delayed reactions appear on subsequent testing with the specific protein. Furthermore, on sensitization to foreign sera, tests in the early stages give delayed, while later tests give immediate, reactions (23).

It would seem then that, in the course of sensitization, both types of hypersensitivity may appear in the same subject and may coexist or be present at different times. Their coexistence has been reported in tuberculin-sensitive humans, in whom tuberculopolysaccharides give immediate reactions and the proteins give delayed reactions (24). In tuberculous and actively sensitized guinea pigs, immediate uterine reactions to tuberculoprotein have been elicited *in vitro*, and anaphylactic shock has resulted from the intravenous injection of tuberculopolysaccharide (25) and tuberculoprotein (26).

A previously undescribed immediate reaction to the injection of tuberculin into BCG-infected guinea pigs has been reported by Dr. A. A. Miles (personal communication), who observed exudation of intravenously injected dye at the test sites within a few minutes. The present writer has confirmed this in guinea pigs (250 to 350 gm.) which had been sensitized with both horse serum and 2 to 3 mg. of killed tubercle bacilli in paraffin oil or by BCG infection.

> After an intravenous injection of 0.50 to 0.75 ml. of a 5 per cent solution of pontamine sky blue (6BX) in normal saline, intradermal injections of 0.10 ml. of solutions of PPD (Weybridge) containing 5, 0.5, and 0.05 mg. per ml. and of 0.10 ml. of horse serum diluted one-tenth in saline were given into the epilated back or the ear tissues. Areas of "blueing," 10 to 20 mm. in diameter, appeared within the first few minutes at positive tests. Control tests in the same and normal animals showed only small slowly developing spots of dye at the injection sites. Systemic and local passive transfer to normal guinea pigs was accomplished successfully, with the serum of guinea pigs sensitized with killed bacilli or BCG and with the serum of rabbits sensitized with killed bacilli. The antibodies which reacted with the PPD could only be shown in this way in the serum of guinea pigs six to eight weeks after sensitization, whereas anti-horse-serum antibodies appeared within two to three weeks. Positive immediate reactions were also obtained in rabbits sensitized with killed bacilli with skin test doses as low as 0.001 mg., after they had been prepared by intravenous injection of 10 ml. trypan blue (0.2 per cent).

In contrast to the findings in guinea pigs sensitized with killed bacilli or BCG, passive transfer of sensitivity was not achieved with the serum of tuberculous guinea pigs infected with virulent H37Rv. This unexplained difference corresponds to the report of Lewis and Seibert (26) that, while the intravenous injection of 15 to 20 mg. of tuberculoprotein produced anaphylaxis in tuberculous

guinea pigs, their serum failed to transfer this hypersensitivity passively. They also found that anaphyactic sensitization was demonstrable only after four weeks from the time of infection.

The evaluation of the relative role of the polysaccharide, protein, and other fractions will depend on the preparation of purer fractions from the tubercle bacillus. Other bacterial polysaccharides have elicited immediate reactions and their proteins have elicited delayed reactions in infected subjects (27*a*, *b*, *c*). In the same way, the intravenous injection of 2 to 3 mg. of tuberculopolysaccharide into sensitized guinea pigs has elicited anaphylactic shock (25). In humans the polysaccharide has provoked an immediate reaction which persisted for two hours and an early reaction which appeared after four hours and persisted for twenty-four hours, at which time parallel tests with tuberculoprotein were becoming manifest (24).

The role of the polysaccharide in an early six-hour reaction to certain tuberculins has been disputed, and it has been claimed that the protein was responsible (28). Nevertheless, it seems that the possibility of an early vigorous reaction to purer polysaccharide preparations, in adequate dosage, merits further study. The high precipitin titer which has been found to polysaccharide in sensitized animals (29) may perhaps mediate an Arthus type of response. Such a reaction, if present, would need to be distinguished from the later delayed reaction attributed to the protein. Interest in this problem is stimulated by the observations of Meynell (30) that the polysaccharide appears to be a surface antigen and the protein a deep antigen in the tubercle bacillus.

PART II. THE SOURCE AND AVAILABILITY OF THE ANTIBODY IN TUBERCULOSIS

Specific Sensitization of Tissue Cells in Tuberculosis

Tuberculin reactions can be elicited in all parts of the body, including the avascular cornea, unlike immediate reactions which only occur in the cornea after it has been vascularized (31). This phenomenon has been taken to support the view that tissues of all types are sensitized in tuberculin hypersensitivity. This assumption has been further supported by many studies in which it has been found that the addition of tuberculin to cultures of tissues made *in vitro* from sensitized animals results in a specific injury to the growing cells (32–36).

In most of these tissue culture experiments, however, the cells examined have been derived mainly from the spleen, lymph nodes, bone marrow, or macrophage exudates; in short, from a restricted range of tissues. Nor have these observations received universal acceptance. Baldridge and Kligman (37) failed to substantiate such findings, and have drawn attention to the technical difficulties inseparable from this method for displaying hypersensitivity states. Similarly, Marks and James (38), in the course of *in vitro* experiments on leukocytes from hypersensitive subjects, found that the addition of tuberculin to suspensions of these cells failed to affect either their oxygen consumption or glucose utilization. Their only specific finding was that the addition of tuberculin resulted in a decrease in the motility of sensitized leukocytes. Nor could they confirm previous reports of toxicity of tuberculin for the leukocytes of hypersensitive subjects *in vivo* (39, 40).

In view of the readiness with which tuberculin reactions can be carried out in the skin, it is significant that tuberculin is apparently devoid of power of injuring tissue cultures of epidermal cells from hypersensitive subjects (41). It seems of interest, too, in this connection that these epithelial cells, cultured under similar conditions, are also insensitive to the action of agents which can excite contact hypersensitivity (42). These findings seem to be in accord with the reports, based on skin-grafting experiments, that the antibodies in the contact type of hypersensitivity come to the skin from below and are not a part of it (43*a* and *b*).

The report that tuberculin is toxic for tissue cultures of hepatic cells from sensitized rabbits is of considerable interest although it is possible that the reticuloendothelial content of this organ may have been responsible (44).

When lymphocytes from tuberculin-hypersensitive subjects are exposed to tuberculin *in vitro*, they are stated to undergo lysis (45, 46). This change becomes evident within twenty minutes, and the observation suggests that such lymphocytes must already be carrying specific antibody. It has also been claimed that the lymphocytes liberate a "sensitizing" factor into the plasma which can render other normal cells susceptible to lysis by tuberculin, although Wesslén (57*c*) was unable to confirm this observation. The lysis by tuberculin of lymphocytes *in vitro* required complement and was not correlated with its inhibitory effect in tissue culture on macrophages or fibroblasts or with the intensity of the skin test reaction. The tissue culture inhibition, however, appeared to be correlated with the skin reactivity and complement was not required (47).

The Passive Transfer of the Tuberculin Reaction

Early attempts to transfer tuberculin hypersensitivity passively by the injection of serum, tissues, or tissue extracts from sensitive subjects gave neither reliable nor consistent results (48). The report by Bail in 1910 (49) that animals injected with suspensions of tissues from tuberculous animals died of delayed tuberculin shock following the intraperitoneal injection of tuberculin twenty-four hours later was dismissed on the grounds that the tissues were toxic in themselves. The more precise and more readily controlled demonstration that this type of hypersensitivity can be transferred by inoculation of cells of reticuloendothelial origin, such as peritoneal macrophages, therefore, represented a marked advance for which the credit must be given to Landsteiner and Chase. Their findings have now been repeated by many others, who have confirmed the observation that both local and general passive transfer of tuberculin and the contact type of hypersensitivity can be achieved by the inoculation of such cells from sensitized subjects (50–55).

In humans, similarly successful transfers of both tuberculin and streptococcal hypersensitivity through the inoculation of circulating leukocytes have been recorded by Lawrence (56*a* and *b*). His findings are of considerable interest, as he claims that this passive sensitization differs in humans from that in experimental animals in two respects. First, the hypersensitive state persists up to two or three months in humans, in contrast to four or five days in experimental animals. Second, successful general passive transfer of hypersensitivity was produced by the injection of leukocytes, which had been disrupted by distilled

water or repeated freezing and thawing, into humans, in contrast to the failure of others to sensitize experimental animals with nonviable or frozen cells (3, 57). Furthermore, he found that passive transfer could be effected in man even after the nucleoprotein sediment in cytoplasmic solution had been treated with desoxyribonuclease or ribonuclease. Lawrence concluded that the passive transfer of tuberculin hypersensitivity did not depend on polymerized desoxyribonucleic or ribonucleic acid components. These are thought to play an important part in antibody synthesis.

These findings pose once again the outstanding problems of the transfer of the delayed type of hypersensitivity by means of lymphoid cells. Are these cells carrying preformed antibody which has attached itself firmly in some way, or are the sensitized cells immunologically activated and capable of handing over the antibody-forming capacity to the cells of the recipient? Some understanding of this fascinating problem may be found in the results of investigations of the following questions. When passive transfer is effected with disrupted cells, is the antibody taken up and demonstrable in the same series of lymphoid cells of the recipient? Does the injection of disrupted cells into the skin produce local sensitization, before the appearance of general sensitization, in the same way as this local sensitization is produced by the injection of viable cell suspensions (52, 57, 59)? Such a study may provide evidence of the presence of preformed antibody, and perhaps of its ability to elicit reactions when free from the specific cells series which carries it.

Of the various types of cell concerned with such passive transfers, the lymphocyte appears to play a major role. Wesslén (*57a, b, c, d*) produced hypersensitivity in guinea pigs by the injection of a suspension of lymphocytes obtained from thoracic duct lymph, and suggested that these cells produce the specific antibody when in the tissues of the recipient. Similar attempts at passive transfer with neutrophil-containing peritoneal exudates, on the other hand, have been unsuccessful (58). Kourilsky, Decroix, and Ganter (59) found that, in such experiments in guinea pigs, the small, mature, lymphocytes formed the majority of the cells in the inoculum and best survived the methods used for preparing suspensions from the spleen and lymph nodes. These writers also transferred passive tuberculin hypersensitivity to guinea pigs with inocula of human leukocytes, and they concluded that the reacting antibody was carried in lymphocytes and that the inflammatory reaction was produced by substances which they liberated. The importance of mononuclear cells in the delayed type of hypersensitivity is indicated further by reports that these cells from cortisone-treated donors can transfer both tuberculin and contact hypersensitivity passively (60, 61).

From the considerable mass of conflicting evidence available, it is too soon to attempt to reach definitive conclusions. The modes of experimentation, the degrees of hypersensitivity of the animals used, and the dosages of tuberculin used have all been widely varied. There would seem as yet little ground for the belief that the parenchymatous cells of an organ are materially sensitized by the procedures used; it appears more probable that the responsive cells are those

of the macrophage group. These latter cells are so widely distributed throughout the body and reach any site of injury so promptly that there would seem to be no inherent difficulty in attributing the ready evocation of tuberculin reactions in different organs to their participation in the response.

Modifications of the source and availability of the antibody: In the passive transfer experiments, it has been found that, allowing for the degree of hypersensitivity of the donor, the degree of hypersensitivity transferred depended on the route of administration and the number of cells employed. The present writer has carried out investigations which appear to influence the antibody in two ways: by decreasing the number of antibody-carrying cells; and by decreasing the local availability of these cells for the purposes of the reactions. These modifications may be brought about by nonspecific and specific methods.

Circulating Leukocytes in the Tuberculin Reaction in the Rabbit

Nonspecific decrease in antibody-carrying cells: Four albino rabbits which had been sensitized by the injection of killed human tubercle bacilli in paraffin oil were irradiated with X-rays four to six weeks later, a single whole-body dose of 250 r being given. The tuberculin reactions became minimal or were absent on subsequent testing. At this time the lymphocytes were markedly reduced. Intradermal injections were given of 0.1 to 0.2 ml. of concentrated suspensions of leukocytes prepared from the buffy coat of the peripheral blood of nonirradiated tuberculin-sensitive rabbits and normal rabbits. These suspensions contained from 80 to 100,000 cells per cu. mm. in the animals own heperinized (75 mg. per ml.) plasma. Superinjection of 0.1 to 0.2 ml. of OT (Human T. (Wellcome Brand) 1:10 and 1:100) immediately afterwards into these cell depots excited vigorous inflammatory reactions at the sensitized cell sites. Normal cells and control injections of sensitized cells also provoked inflammatory nodules, but these were far smaller and lacked the spreading induration and edema produced by the reaction of the sensitized cells to the tuberculin.

It has been suggested that X-irradiation inhibits the tuberculin reaction in rabbits by an adrenocortical "stress" response, and not by its effect on lymphoid tissues (62). Lymphoid tissue and lymphocytes are however highly sensitive to ionizing irradiation not only *in vivo*, but *in vitro* as well (63). The inhibiting effects of irradiation may therefore be due in part to the nonspecific decrease in lymphoid tissue and circulating lymphocytes. Further information regarding the relationship of lymphoid regeneration and the reappearance of circulating lymphocytes to the tuberculin reaction is desirable. In addition, little is known regarding the immunologic capacity of lymphoid tissue during regeneration after irradiation.

Specific decrease of circulating lymphocytes: The intravenous injection of PPD into tuberculin-sensitive rabbits produces a specific lymphopenia (64). Tuberculin tests carried out twelve hours after the PPD was injected were much decreased in size, although it was considered that they were more intense, and it was suggested that an enhancing factor may have been liberated from lymphocytes lysed by tuberculin (65).

The interpretation of these results is open to question.

The present writer has repeated these experiments in 9 albino rabbits (3.5 to 4 kg.) which had been sensitized by injection of killed tubercle bacilli. Six weeks later intravenous injections of 0.10 mg. of PPD (Weybridge) were given. These injections were regularly followed by a lymphopenia, most marked six to nine hours later, followed by a return to normal, and sometimes above normal, absolute numbers after eighteen to twenty-four hours. In this respect the observations confirmed the first of the reports (64). Tuberculin tests were carried out at the same time as the intravenous injection of PPD and at intervals of three hours thereafter. The initial tests were greatly decreased and were frequently completely inhibited. The reactions at successive tests became larger, with a return to the original size after one to two days, and on occasion only later. The reactions were enhanced in speed of appearance, but they did not show any enhancement of induration or inflammation. There seem to be better grounds for regarding the effect of the intravenous injection of PPD as inhibitory in nature, and the results suggest a relationship between the reactions elicited and the level of the circulating lymphocytes.

Neutrophilic cells in the tuberculin reaction: Many attempts have been made to determine, by histologic methods, the role in tuberculin reactions of the different types of infiltrating cell. The reports that mononuclear cells predominate in the early stages of weaker reactions (66, 67) have been disputed and it has been claimed that neutrophils predominate at this stage (68). Laporte (67) and Gell and Hinde (69) have found after careful study that the incidence of neutrophilic cells varies according to the layer of tissue examined. Gell and Hinde describe the cellular infiltration as a local lymph-node, reticulo-endothelial type of response. The early and often intense outpouring of neutrophilic cells in reactions to tuberculin and tubercle bacilli (70–73) has led Rich (74) to suggest that it cannot be regarded as purely nonspecific and unrelated to the tubercle bacillus. In the present paper, evidence has been given suggesting that the mononuclear cell is the source of antibody, and there are in addition reports suggesting that the early mononuclear cellular infiltration is derived from circulating leukocytes (75).

The part of the neutrophilic cell in the tuberculin reaction in the rabbit has been studied by the present writer by carrying out tuberculin tests in 6 sensitized albino rabbits after they had received intravenous injections of nitrogen mustard (76). This was injected in a dose of 1.75 mg. per kg., and was followed by a profound neutropenia, so that on the third and fourth days from 83 to 95 per cent of the circulating cells were lymphocytes. Tuberculin skin tests remained positive during this period of neutropenia, and histologic examination showed a virtual absence of infiltrating neutrophilic cells; mononuclear cells predominated overwhelmingly from the first few hours. These experiments also demonstrated some relationship between the number of circulating lymphocytes and the reaction to tuberculin. For the first two days after its injection, the nitrogen mustard produced a nonspecific decrease in the circulating lymphocytes which fell below the neutrophile cells. During this time tuberculin tests excited smaller reactions than were obtained with tests in the next two days, when the number of lymphocytes rose and exceeded the neutrophile cells, as described above.

There is also an earlier report that the intraperitoneal injection of tuberculin into sensitive animals, after they had been deprived of their neutrophilic cells by treatment with benzole, excited a mononuclear cellular exudate (77). It would seem, therefore, that depression of the circulating lymphocytes is associated with inhibition of the tuberculin reaction, whereas the reaction persists in spite of suppression of the neutrophils.

Circulating leukocytes in the tuberculin reaction in man: The importance of circulating leukocytes in the initiation of the tuberculin reaction in humans is suggested by certain of the present writer's findings (12) which support the conclusions drawn from the studies in the rabbit.

> It was found that the superinjection of 0.05 to 0.10 ml. of epinephrine hydrochloride (1:1,000) into Mantoux tests, up to at least three hours after the tuberculin inoculation, inhibited the reaction completely, when control tests carried out at the same time produced reactions of 80 to 130 sq. mm. in size. Biopsy specimens of control and epinephrine-inhibited reactions removed three and twenty-four hours after testing showed a striking decrease in the cellular infiltration at the epinephrine-treated sites.

It is suggested that the slowing of access of circulating leukocytes into the area, and in particular of the antibody-carrying lymphocytes, had prevented the reaction. Local or resting reticulo-endothelial cells would appear to participate in the later stages of the reaction rather than in its initiation.

Local epinephrine injection has therefore a paradoxical effect on tuberculin test reactions which requires explanation (12). It is suggested that the behavior of the antigen is modified in the patch test, whereas the inhibition of the Mantoux test results from modification of the antibody.

The behavior of the antigen is influenced both by the method of testing and by time relationships. Thus, in the patch test there is no tuberculin in the tissues at the commencement of the test, and its concentration rises as it penetrates the skin. The decreased circulation at the test site, which results from the epinephrine-produced vasoconstriction, must in turn decrease lymphatic absorption and lead to retention of the tuberculin locally. The higher local concentration is manifested by an enhanced reaction.

It might be expected that decreased lymphatic absorption would lead to retention of the tuberculin in Mantoux tests as well. In the Mantoux test, however, the local concentration of tuberculin is highest at the outset and can only decrease during the period of vasoconstriction. The lymphatic absorption from the intracutaneous tissues is greater than from the epidermis, and this too would mitigate the degree of local retention in the epinephrine-treated Mantoux test, as compared with the patch test. It has already been pointed out that local epinephrine was most effective in inhibiting reactions of moderate intensity, that is, reactions more likely to be affected by a fall in the local concentration of tuberculin. Whereas these factors may influence the local concentration of tuberculin, it is suggested that the inhibition of the Mantoux test is due chiefly to decreased availability of antibody-carrying cells during the period of vaso-

constriction. Clinical correlations between the peripheral vascular responses and tuberculin reactions have been reported; vasoconstriction was found to be associated with decreased reactions and vasodilatation with increased reactions (78). Decreased skin reactivity to whealing agents has also been found to parallel decreased tuberculin reactions (79).

Macrophages and circulating leukocytes in tuberculin reactions in the guinea pig: The influence of macrophages and circulating leukocytes was studied by the writer in 7 sensitive guinea pigs in which the tuberculin reaction had been inhibited by prior systemic administration of an antihistaminic drug, promethazine (Phenergan®). The intramuscular injection of massive doses, such as 50 mg. per kg. daily for one and one-half to two days before testing, inhibited previously necrotic reactions to a marked degree, and weaker reactions frequently failed to appear.

These findings confirm a previous report by Sarber (80). Previous contradictory reports on the effect of systemic administration of these drugs may have been due to inadequate pretreatment (81, 82).

Intradermal injections of 0.10 to 0.20 ml. of concentrated suspensions of peritoneal macrophages and circulating leukocytes, derived from tuberculin-sensitive and normal guinea pigs, were given into the epilated backs of promethazine-treated animals. The cell suspensions contained from 80 to 100,000 cells per cu. mm. in the animal's own heparinized (75 mg. per ml.) plasma. Superinjections of 0.10 to 0.20 ml. of OT, 1:10 (B. W.), and of the same volume of the vehicle, were given into these cell deposits. The reactions at tuberculin-injected sensitized cell sites were more vigorous, larger than those at other sites in 5 of 7 animals, and showed spread of induration and edema. The cell deposits themselves provoked nodular reactions, such as those described elsewhere (52, 59), without the spreading induration of positive test reactions.

As the promethazine did not decrease the circulating lymphocytes, it is suggested that its effect on tuberculin reactions was due to the inhibitory action which antihistaminic drugs exert on vascular responses to specific and nonspecific agents (83, 84). Such a nonspecific inhibition of the tuberculin reaction may have resulted from either of two effects or both. Decreased access of circulating leukocytes to the test site may have modified the availability of antibody-carrying cells; or the inflammatory response to the tuberculin reaction may have been diminished by the decreased vascular responses. Histologic examination of tuberculin tests from guinea pigs before and during the administration of promethazine showed a considerable decrease in edema, which was easily seen macroscopically. Cellular infiltrations were so marked in both instances that no conclusions could be drawn from them. It seems that the inhibition was not due solely to an anti-inflammatory action of the promethazine, since the introduction of specific reacting cells into the skin together with tuberculin provoked a vigorous inflammatory response.

PART III. INFLAMMATORY RESPONSE OF THE TISSUES TO THE TUBERCULIN REACTION

Corticotropin and cortisone are the most important agents capable of decreasing nonspecific and specific inflammatory reactions (85, 86, 87). The

mechanism of their action is still obscure, and they produce effects in a number of ways and at a number of differing points. Long and his colleagues have made closely reasoned investigations into the actions of cortisone on tuberculin sensitivity. They have proceeded from the study of the influence of thyroid and adrenal hormones (88) to the role of hormonal and dietary factors and ascorbic acid (89*a*, *b*, *c*). Their observations have led them to identify organic phosphates such as glucose-1-phosphate as immediate factors responsible for the decreased tissue reactivity, even when administered to guinea pigs in very small doses (90). Long (91) concludes that cellular permeability is modified by cortisone, thereby interfering with the liberation of toxic materials.

Without necessarily being immediately responsible for decreasing tuberculin reactions, the other factors modified by cortisone may also be playing a part. The role of lymphoid tissue and of circulating lymphocytes has been discussed earlier in this paper. Agents which decreased these tissues in amount and availability, either nonspecifically or specifically, produced decreased reactions. A reduction of lymphocytes and lymphoid tissue by cortisone is a regular feature, and Gell and Hinde (69), in a careful study of cortisone-inhibited tuberculin reactions in the rabbit, have found a decrease in the mononuclear cellular infiltration. These cells are derived, in the early stages at least, from the circulating leukocytes.

It is possible that the nonspecific lymphopenia which cortisone produces may be responsible in part for its inhibitory effect on the tuberculin reaction in the same way as the lymphopenia which follows X-irradiation or intravenous PPD is associated with decreased reactions. Cortisone also exerts potent effects on blood vessels and vascular responses, raising the threshold of capillary permeability (92), enhancing vasoconstrictor tone, and reducing endothelial damage in reactions to tubercle bacilli (93, 94). The influence of vascular responses in tuberculin reactions has been discussed, and the cortisone-induced effects in turn may modify both the initiation of the reaction and the inflammation which succeeds the combination of antigen and antibody.

The depression of tuberculin sensitivity by sphingosine (95) and by certain antituberculous surface-active agents (96) is obscure and may be related to some depression of cellular or tissue reactivity.

DISCUSSION

The preceding observations indicate certain of the antigen-antibody relationships which are necessary for the development of tuberculin reactions in sensitive subjects. In order that a reaction should appear after the introduction of the antigen, tuberculin, it must persist locally in an adequate concentration for a sufficient period of time. The local persistence of the tuberculin, which can be modified considerably, depends upon the rate of lymphatic absorption which, in turn, is influenced by local vascular responses. Increased lymphatic absorption results in decreased reactions and vice versa, depending on the method of testing.

The study of the specific reacting antibody has been assisted by the identifi-

cation of reticulo-endothelial tissue, such as macrophages, and particularly of lymphoid tissue and circulating lymphocytes as a source of antibody as, for example, in passive transfer experiments. Furthermore, decreased or inhibited tuberculin reactions result from treatment with agents which reduce lymphoid tissue and lymphocytes. The reduction may be produced nonspecifically, i.e., by X-irradiation, or specifically, i.e., by the intravenous injection of tuberculoprotein. The suggestion that circulating cells, especially circulating lymphocytes, participate in the initiation of the tuberculin reaction in humans is supported by the absence of infiltrating cells in Mantoux tests which have been inhibited by the local superinjection of epinephrine (12). The appearance of tuberculin reactions in experimental animals rendered neutropenic by treatment with nitrogen mustard indicates that the neutrophilic cell is not a major participant in the reaction. Enhanced, neutrophilic infiltration may well be an indication of a hypersensitive tissue reaction rather than a factor in its initiation.

There are reports of the behavior of sensitized mononuclear cells in active infection with tubercle bacilli which are of interest in relation to the above findings. Lurie (97) found that mononuclear cells are mobilized with greater rapidity in response, not only to tubercle bacilli, but also to nonspecific inflammatory stimuli. In addition, the injection of macrophages containing ingested tubercle bacilli into the anterior chamber of the rabbit's eye produced a rapidly progressive infection if the cells were derived from normal animals, and a much retarded infection with cells from sensitized animals (98). More recently Suter (99) has reported that tubercle bacilli multiplied inside normal macrophages in tissue culture, whereas their growth was retarded in sensitized cells.

Discussion of the value to the host of having phagocytic cells, which are also a source of specific reacting antibody, falls into the controversy regarding the role and distinction of hypersensitivity and immunity. There are many deficiencies in our knowledge of this problem and it will not be discussed here. Controversies around the source and development of mononuclear cells have also not been entered into, important though they may be. It was believed that the various types of mononuclear cell referred to have certain common features, providing, for example, a source of specific antibody for passive transfer. Studies of their function under special circumstances may clarify some of the morphologic problems.

Although blood vessels are a shock organ in anaphylactic hypersensitivity, they do not behave in the same way in tuberculin hypersensitivity. Nevertheless, vascular responses participate in the initiation of the tuberculin reactions by influencing the local persistence of the tuberculin. The vascular responses also participate by acting as a "via media" for a source of reacting antibody, namely the circulating lymphocytes. The effect of altered vascular responses depends on the type of test employed, for decreased circulation produced by local epinephrine enhances the patch test by modifying the persistence of antigen, whereas it inhibits the Mantoux test by modifying the availability of antibody. Thus the same vascular response can produce opposite end effects. By the same token, agents with opposing vascular actions can also produce the same end effect.

Histamine superinjection, on the one hand, inhibits the Mantoux test by "flushing away" the antigen; on the other hand, local epinephrine inhibits the Mantoux test by influencing the antibody. In the study of agents or circumstances which modify tuberculin reactions these mechanisms seem to merit consideration.

Tuberculin can provoke reactions in concentrations which are not toxic to the tissues, and apparently it is not toxic to epidermal tissues of sensitized subjects (41). Hence, it is necessary to search for a stimulus capable of initiating the reaction. This is particularly so in the patch test in which not even trauma is inflicted. The early reaction to tuberculoprotein in actively sensitized animals, which has been described earlier, is manifested by increased capillary permeability. This may assist in the initiation of the reaction by aiding the passage of circulating antibody-carrying cells into the tissues. The "fixation" of tuberculin in human tissues within the first ten minutes after its introduction may be related in some way to this early reaction, although nonspecific fixation may be equally rapid.

In all of the studies with mononuclear cells, their number and availability have been shown to influence the extent of the reaction to the tuberculin. This suggests that the delayed nature of the tuberculin reaction may be due to the time required for the appearance of sufficient numbers of antibody-carrying cells at the test site where, after reacting with the tuberculin, an inflammatory response is excited. Kourilsky, Decroix, and Ganter (59) have arrived at a similar conclusion, although they consider that the lymphocytes produce the antibody in the recipient after their injection. Favour's report (45) that lymphocytes are lysed *in vitro* by tuberculin within twenty minutes suggests that they are either carrying already formed antibody or produce it rapidly. Lawrence's claim to successful passive transfer with disrupted cells even after treatment with desoxyribonuclease or ribonuclease (56c) corresponds with Favour's report that sensitized lymphocytes liberate substances which can sensitize normal cells against tuberculin. As there appears to be an intimate relationship between the reacting antibody and the intact lymphoid cells, it remains to be seen whether such a relationship is essential for the production of the tuberculin reaction, or whether preformed antibody can fulfill this function without being taken up by specific lymphoid cells.

SUMMARY

In the investigations of antigen-antibody relationships in the initiation of the tuberculin reaction, it was observed that both antigen and antibody were subject to modifications of a nonspecific and specific nature which, in turn, influenced the reactions obtained. These mechanisms should be considered in evaluating the influence of other agents on tuberculin reactions.

Antigen: In man, the local persistence of tuberculin is influenced by lymphatic absorption. When absorption is decreased, reactions are enhanced and vice versa. Local injection of histamine and epinephrine were used to demonstrate this phenomenon.

"Fixation" of the tuberculin appears to commence within the first ten

minutes after injection and is completed for a moderate dose of tuberculin within one or two hours. These times were arrived at by studying the modified reactions resulting from the superinjection of saline, hyaluronidase, and histamine into Mantoux tests.

Tuberculoprotein excites an immediate reaction on injection into sensitive guinea pigs and rabbits which is manifested as an increase in capillary permeability.

Antibody: Reactions to tuberculin are inhibited in sensitive animals by agents which reduce lymphoid tissue and circulating lymphocytes nonspecifically, i.e., by X-rays or, specifically, by the injection of tuberculoprotein.

Adequate antihistaminic treatment inhibits tuberculin reactions in guinea pigs by modifying vascular responses in a nonspecific manner.

Local passive transfer tests with homologous circulating leukocytes or macrophages gave positive reactions in animals in which inhibition had been produced, as in the above two instances. This suggests that the inhibitory agents have modified the number or availability of circulating leukocytes, and especially circulating lymphocytes, and have therefore modified the source of reacting antibody concerned in the initiation of the reaction.

Tuberculin reactions were obtained in rabbits treated with nitrogen mustard so as to suppress their circulating neutrophilic cells, thus indicating that these cells are not essential for the initiation of the reaction.

In man the injection of epinephrine into Mantoux tests inhibited the reactions, and this was associated with a decrease in the infiltration of circulating leukocytes. It is suggested that the absence of circulating lymphocytes is responsible for the inhibition.

It is suggested also that the time required for the arrival of sufficient numbers of antibody-carrying lymphocytes to combine with the antigen and thus to excite an inflammatory response may be one of the reasons for the delayed nature of the reaction.

SUMARIO

La Relación entre Factores Anespecíficos y Específicos en la Reacción a la Tuberculina: Repaso

En las investigaciones de las relaciones de antígeno-anticuerpo en la iniciación de la reacción tuberculínica, observóse que tanto antígeno como anticuerpo son susceptibles de modificaciones de naturaleza anespecífica y específica, que a su vez afectan las reacciones obtenidas. Hay que considerar esos mecanismos al justipreciar el influjo de otros agentes sobre las reacciones a la tuberculina.

Antígeno

En el hombre, la persistencia local de la tuberculina se ve afectada por la absorción linfática. Cuando ésta disminuye, las reacciones se acentúan, y viceversa. Para demostrar este fenómeno, se usó la inyección local de histamina y de epinefrina.

La "fijación" de la tuberculina parece comenzar dentro de los primeros diez minutos consecutivos a la inyección, alcanzando su término en una o dos horas con una dosis moderada de tuberculina. Se obtuvieron esos valores estudiando las reacciones modificadas producidas por la sobreinyección de solución salina, hialuronidasa e histamina en las pruebas de Mantoux.

Al ser inyectada en cobayos y conejos sensibles, la tuberculoproteína excita una reacción inmediata, que se traduce por aumento de la permeabilidad capilar.

Anticuerpo

Las reacciones a la tuberculina son inhibidas en los animales sensibles por agentes que reducen el tejido linfoideo y los linfocitos circulantes, anespecíficamente como con los rayos X, o específicamente como con la inyección de tuberculoproteína.

Un tratamiento antihistamínico adecuado inhibe las reacciones tuberculínicas en los cobayos por modificar las respuestas vasculares en forma anespecífica.

Las pruebas de transferencia pasiva local con leucocitos o macrófagos circulantes homólogos dieron reacciones positivas en los animales en que se había producido inhibición, como en los dos ejemplos citados. Esto sugiere que los agentes inhibidores han modificado la cantidad o la accesibilidad de los leucocitos, y en particular los linfocitos, circulantes, modificando así la fuente de los anticuerpos reactores que intervienen en la iniciación de la reacción.

Obtuviéronse reacciones tuberculínicas en conejos tratados con mostaza nitrogenada con mira a suprimir los neutrófilos circulantes, indicando esto que no son indispensables esos glóbulos para la iniciación de la reacción.

En el hombre, la inyección de epinefrina en las pruebas de Mantoux inhibió las reacciones, lo cual se asoció con disminución de la infiltración por leucocitos circulantes. Sugiérese que la ausencia de linfocitos circulantes es la causa de la inhibición.

Sugiérese que el tiempo requerido para la llegada de linfocitos cargados de anticuerpos en cantidades suficientes para combinarse con el antígeno y excitar así una respuesta inflamatoria, puede constituir una de las causas de la naturaleza retardada de la reacción.

RESUME

Rapports entre les facteurs spécifiques et non spécifiques dans la reaction à la tuberculine: Un exposé

Dans les investigations concernant les rapports antigène—anticorps à l'origine de la réaction à la tuberculine, il fut observé que l'antigène et l'anticorps étaient tous deux sujets à des modifications de nature spécifique et non spécifiques, qui, à leur tour, influençaient les réactions obtenues. Ces mécanismes doivent être considérés dans l'évaluation de l'influence exercée par d'autres agents dans les réactions à la tuberculine.

Antigène

Chez l'homme, la persistance locale de la tuberculine est influencée par l'absorption au niveau des lymphatiques. Quand l'absorption est réduite, les réactions s'accroissent et vice versa. L'injection locale d'histamine ou d'adrénaline a été utilisée pour démontrer ce phénomène.

La "fixation" de la tuberculine paraît débuter dans les premières dix minutes qui suivent l'injection et s'achever en une ou deux heures avec une dose moyenne de tuberculine. Ces déterminations ont été obtenues par l'étude des réactions modifiées des tests de Mantoux à la suite d'une "superinjection" de sérum physiologique, d'hyaluronidase et d'histamine.

La tuberculo-protéine provoque une réaction immédiate à l'injection chez le cobaye et le lapin sensibles; il se produit un accroissement de la perméabilité capillaire.

Anticorps

Les réactions à la tuberculine sont inhibées chez l'animal sensible par les agents qui provoquent la réduction du tissue lymphoïde et des lymphocytes circulants, soit non spécifiquement comme par les rayons X, soit spécifiquement comme par l'injection de tuberculoprotéine.

Un traitement antihistaminique adéquat inhibe les réactions à la tuberculine chez le cobaye, par une modification non spécifique des réactions vasculaires.

Les tests de transfert local passif, par les leucocytes homologues ou les macrophages circulants, donnaient des réactions positives chez l'animal ayant été soumis aux procédés d'inhibition, comme dans les deux cas ci-dessus. Ces faits suggèrent que les agents inhibiteurs, en modifiant le nombre ou la capacité de disposer des leucocytes circulants, et en particulier des lymphocytes, modifiaient également la source d'anticorps intéressés dans l'instauration de la réaction.

Les réactions à la tuberculine furent obtenues chez des lapins traités par la moutarde-azote, dans le but de supprimer les neutrophiles circulants, démontrant ainsi que ces cellules n'étaient pas indispensables à l'instauration de la réaction.

Dans les tests de Mantoux chez l'homme, l'injection d'adrénaline inhibait la réaction, et ce phénomène s'accompagnait d'une réduction de l'infiltration par les leucocytes circulants. L'auteur suppose que c'est cette absence de lymphocytes circulants qui est responsable de l'inhibition.

Le temps nécessaire à l'arrivée d'un nombre suffisant de lymphocytes porteurs d'anticorps susceptibles de se combiner à l'antigène, et de provoquer ainsi la réaction inflammatoire, est considéré comme une des raisons du retard de la réaction.

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THE CULTURAL CHARACTERISTICS AND ANIMAL PATHOGENICITY OF AN ATYPICAL ACID-FAST ORGANISM WHICH CAUSES HUMAN DISEASE[1, 2, 3]

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INTRODUCTION

Two cases of human disease caused by an atypical acid-fast organism have been reported previously (1) from this laboratory. In the first case, the organism was repeatedly recovered from sputum cultures of a man who had a disease clinically resembling pulmonary tuberculosis. The organism was demonstrated in the lung tissue removed surgically and was cultured from the pathologic lesions. In the second case, an identical organism was recovered from a draining inguinal sinus which developed following surgical removal of a lymph node, was subsequently cultured from caseous lesions found at post-mortem examination, and was demonstrated in the tissues by acid-fast stains. All attempts to recover typical tubercle bacilli in these two cases, including cultures and direct animal inoculations of infected tissues, were unsuccessful.

This organism has been called the "yellow bacillus." It is acid-fast with the Ziehl-Neelsen and Fite stains (2, 3), is approximately three times the length of the H37Rv strain of the human tubercle bacillus, and does not produce progressive disease in guinea pigs.

The cultural characteristics of this organism have been reported previously in detail (1). It grows rapidly on the ordinary media used routinely for the isolation of *Mycobacterium tuberculosis* and can also be grown, but with difficulty, on blood agar and Sabouraud's agar, and in infusion broth. The organism grows slowly at approximately 22°C. and more rapidly at 37°C. Colonies are usually smooth and moist. The color varies from cream to yellow or yellow-orange, with the yellow color becoming more pronounced when grown at room temperature. As the culture ages and the medium loses its moisture, the colonies may lose their smooth, glistening appearance and become dry and granular. In modified Proskauer and Beck liquid medium (4), heavy growth occurs at the bottom of the tube with occasional formation of a pellicle. Homogeneous dispersion can be achieved by vigorous shaking. The growth is most characteristic and distinctive when grown on Steenken and Smith's solid medium (5), because the blue background of the medium contrasts with the yellow color of the colony.

Initial studies of the pathogenicity for animals of the two strains of the "yellow

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bacillus" previously reported and of a third strain recently isolated constitute the basis of the present report.

METHODS

Broth cultures of the "yellow bacillus" were inoculated into guinea pigs, golden hamsters, rats, mice, rabbits, and chickens. Skin-sensitivity tests were performed on the rabbits, chickens, and guinea pigs four and eight weeks later. The guinea pigs were tested in the skin of the abdomen, the rabbits in the skin of the ear, and the chickens in the skin of the wattle. Each animal was tested with 0.1 ml. of a 1:10 dilution of Wyeth's Old Tuberculin, 0.04 ml. of full strength Jensen-Salsbery avian tuberculin, 0.1 ml. of a 1:10 dilution of homologous vaccine from the "yellow bacillus," and 0.1 ml. of a broth control. The homologous vaccine was produced by a four-month growth of the original two strains of the "yellow bacillus" in 500 ml. of liquid medium (modified Proskauer and Beck) in vaccine bottles incubated in a flat position. The cultures were then autoclaved at 17 pounds of pressure for forty-five minutes, filtered through a Seitz filter, and concentrated in a calcium chloride desiccator. The broth control consisted of the culture medium.

OBSERVATIONS

Pathogenicity for Normal Guinea Pigs

Twenty-five guinea pigs were inoculated subcutaneously with 0.5 ml. of a broth culture of the "yellow bacillus." A local lesion appeared at the site of inoculation four days to two weeks later and gradually enlarged during the next one to two weeks. This lesion was characterized by a central zone of necrosis surrounded by a zone of granulomatous tissue. The necrotic material consisted of amorphous debris in which many nuclear fragments and numerous large atypical acid-fast organisms were seen. The granulomatous tissue consisted of numerous epithelioid cells, occasional giant cells, and a few polymorphonuclear leukocytes. Many of these lesions ulcerated spontaneously, approximately two to four weeks after inoculation, and discharged yellow pasty material. In the absence of ulceration, the lesions receded spontaneously. Ulceration in some animals appeared within a few hours after skin testing. The regional lymph nodes usually showed granulomas, which in some instances became necrotic (Plate I, figure 1).

The animals were sacrificed at the end of two, four, six, eight, and sixteen weeks. The majority of the animals autopsied at the end of the second and fourth weeks showed no evidence of visceral disease grossly, but showed a few microscopic granulomas in the lungs, liver, spleen, and abdominal lymph nodes. Cultures from the site of inoculation yielded a heavy growth of the "yellow bacillus." Irrespective of whether microscopic lesions were demonstrable, the "yellow bacillus" usually could be recovered in small numbers by culture of the liver and spleen. Sometimes an occasional acid-fast organism could be seen in tissues in which no microscopic lesions were seen. Most of the animals sacrificed at the end of six and eight weeks failed to show visceral lesions either grossly or microscopically. A few of the animals were allowed to survive for four months. Neither visceral nor local lesions were found.

Pathogenicity for Guinea Pigs Given Cortisone

Ten guinea pigs were injected intraperitoneally with 0.5 ml. of a broth culture of the "yellow bacillus" and were also given 10 mg. of cortisone twice each week.

Three animals were sacrificed at the end of four weeks, 3 at eight weeks, one died spontaneously at the end of eleven weeks, and the remaining 3 were sacrificed at the end of twenty weeks. None of the animals showed gross evidence of disease except the animal which died spontaneously. This animal had a pleurisy.

On microscopic examination, one of the 3 animals which were sacrificed at the end of four weeks showed no disease; the other 2 showed several granulomas in the lungs. These granulomas consisted of poorly outlined accumulations of macrophages. The lesions seemed to be arising chiefly in the interstitial tissue of the lung but extended into the alveoli. No necrosis was observed. An occasional acid-fast organism was found in the lesions.

The 3 animals which were sacrificed at the end of eight weeks showed numerous granulomas in the lungs. In addition, one of them showed several granulomas in the center of the malpighian corpuscles in the spleen. Occasional organisms were found in the lesions.

The one animal which died spontaneously at the end of eleven weeks showed a moderate number of granulomas in the lungs and a diffuse purulent bronchopneumonia considered to be due to a secondary invader. Occasional acid-fast organisms were found in the granulomas. This animal also presented several large granulomas in the liver which were partly necrotic and partly calcified and in which occasional organisms were seen. A similar lesion was found in the capsule of one kidney.

The 3 remaining animals were sacrificed at the end of twenty weeks. One showed an occasional granuloma in the lungs and hyperplasia of the lymph nodes; one showed only pulmonary granulomas; and the third showed no lesions.

There was less disease in the animals sacrificed at the end of twenty weeks than in those sacrificed earlier. Cultures of the tissues showed no growth. Eight of the 10 guinea pigs were skin tested at the end of four weeks according to the technique previously described. The surviving animals were retested at the end of eight weeks. No reactions to the test substances were observed.

TABLE 1

EFFECT OF CORTISONE ON THE DEATH RATE OF HAMSTERS INOCULATED WITH THE "YELLOW BACILLUS"

Weeks After Inoculation	21 Not Injected with Cortisone		21 Injected with Cortisone	
	Sacrificed	Died	Sacrificed	Died
1	0	1	0	0
2	0	3	0	3
3	0	1	0	4
4	4	1	0	7
5	0	0	0	1
6	4	1	0	5
7	0	0	0	0
8	1	2	0	0
9	0	3	0	0
10	0	0	0	1
11	0	2	0	0

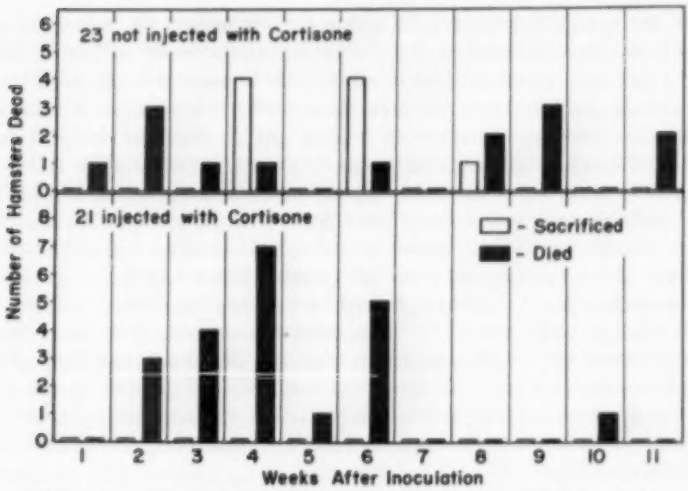

FIG. 1. Effect of cortisone on the death rate of hamsters inoculated with the "yellow bacillus."

Pathogenicity for Normal Hamsters

Forty-four Syrian golden hamsters were inoculated intraperitoneally with 0.5 ml. of a broth culture of the "yellow bacillus." Of these, 21 received 7.5 mg. of cortisone subcutaneously twice each week; the other 23 animals did not receive cortisone. In the group of animals not injected with cortisone, 9 were sacrificed and the others died spontaneously (table 1 and figure 1).

One animal died within twenty-four hours after inoculation and showed intraperitoneal hemorrhage secondary to perforation of the liver which was due to needle puncture during the process of inoculation. Microscopically, organisms were present in the needle tract and in the abdominal lymph nodes. In the other animals, the gross post-mortem findings (Plate I, figure 2) were relatively uniform irrespective of the length of survival. There was frequently an exudate in the left upper quadrant. Later this tended to be replaced by fibrous adhesions. In all animals, the omentum and mesentery were thickened and there was extensive involvement of the mesenteric lymph nodes, peripancreatic lymph nodes, spleen, and liver; quite frequently, the genital tract, the thoracic lymph nodes, and thymus. Involvement of the retroperitoneal fat produced perirenal lesions extending into the capsule of the kidney.

Microscopically, the most characteristic lesion was the massive involvement of the abdominal lymph nodes. This involvement consisted of granulomas (Plate I, figure 3) showing extensive proliferation of macrophages with destruction of the nodal architecture. In the early stages, there were moderate numbers of polymorphonuclear leukocytes in the granulomatous lesions. In the lesions of animals which survived for a longer period of time, occasional giant cells were found. The granulomatous process extended into the surrounding fat and in-

volved the interstitial pancreatic tissue by contiguity. Occasionally, isolated lesions were demonstrated in the pancreatic parenchyma (Plate I, figure 4). The lymph nodes about the fore-stomach were frequently involved. The gastrointestinal tract in several of the animals showed granulomas in the mucosa and beneath the serosa. A characteristic finding was involvement of the tissue about the genital tract. The diaphragm was frequently affected. The spleen (Plate II, figure 5) invariably presented extensive granulomatous lesions which began in the malpighian corpuscles and later destroyed the splenic architecture. The cellular components of these lesions were similar to those seen in the lymph nodes. The liver showed numerous small granulomas located primarily in the portal areas and beneath the capsule. In only an occasional animal did the hepatic lesions become large enough to form confluent nodules. The lungs (Plate II, figure 6) contained small granulomas beneath the pleura and throughout the parenchyma, most numerous about the bronchi. The pulmonary involvement occasionally consisted of an interstitial reaction in the alveolar walls. Granulomas were sometimes found in the bone marrow. The renal parenchyma and the heart were rarely involved.

In approximately 25 per cent of the animals, some of the lesions showed necrosis which varied in extent from minute foci occupying the central portion of a granuloma to massive destruction. The areas of necrosis showed fragmented nuclei rather than the amorphous eosinophilic debris seen in caseation necrosis in human tuberculosis. In addition to numerous macrophages, polymorphonuclear leukocytes were frequently found in small numbers at the periphery of such lesions.

In 80 per cent of the animals which survived beyond the fourth week, small rounded calcific deposits were observed in the necrotic and granulomatous lesions. In animals which survived for a longer period of time, these flecks become aggregated into larger masses (Plate II, figure 7). The outermost rim of these deposits contained calcium phosphate as indicated by Von Kossa's stain (6).

With Fite's acid-fast stain, numerous large atypical acid-fast organisms were demonstrated in the granulomatous lesions (Plate II, figure 8). Frequently, hundreds of organisms in a single field could be seen with the high dry objective (×450). Ordinarily they were most numerous in the lesions in the lymph nodes and spleen; the lesions in the liver and lungs contained fewer organisms. Sometimes, particularly in the lungs, organisms could be found in small numbers in the tissues in which no lesions were demonstrable.

Pathogenicity for Hamsters Given Cortisone

Twenty-one hamsters were given cortisone. All of these animals died during the experiment (table 1 and figure 1); none were sacrificed. The cortisone-injected animals presented gross post-mortem findings which were similar to the findings in the group not injected with cortisone, except for the fact that the animals given cortisone had a fibrinous exudate within the abdominal cavity and smaller individual lesions in the viscera.

Histologically, a more striking difference was observed. The distribution of the

lesions was similar to those in the animals not given cortisone, but the lesions tended to be smaller. Some of the lesions in 72 per cent of the animals showed central necrosis, or were composed entirely of necrotic tissue, but the cellular reaction was less extensive (Plate III, figure 9). In the remaining animals, the granulomatous lesions were indistinguishable from those in the animals not given cortisone. Calcification of the lesions occurred infrequently in the animals surviving four or more weeks. In 2 of the animals, thrombosis occurred in a pulmonary vein and in its demonstrable tributaries. Small foci of necrosis were found in the walls of these vessels.

Acid-fast stains of the tissues (Plate III, figure 10) revealed many more organisms in the granulomatous and in the necrotic lesions in this group of animals than in the lesions of the animals not injected with cortisone. It was not uncommon to find a heavy concentration of organisms around the granulomatous periphery of necrotic foci, while in the central areas only a few organisms were present. Furthermore, the organisms in the central areas frequently were of smaller size, showed more beading and fragmentation, and were not as intensely acid-fast as the larger organisms at the periphery. In the 2 animals in which necrosis and thrombosis of a pulmonary vein had occurred, organisms were demonstrated in moderate numbers within the necrotic lesions and in smaller numbers within the thrombi. Organisms were present in tissues of some animals in which no lesions could be found.

Cultures from the liver, spleen, and lungs, and from other tissues when grossly involved almost invariably yielded a heavy growth of the "yellow bacillus" irrespective of whether or not the animals from which they were taken were given cortisone.

Some of the hamsters injected with cortisone developed nodules in the subcutaneous tissue which measured up to 2 mm. in diameter. These nodules contained white pasty material. Microscopically, this material consisted of amorphous debris with no surrounding cellular reaction. A few atypical acid-fast organisms were demonstrated in the amorphous debris. These nodules probably consisted of residual cortisone and its vehicle in the tissues.

Pathogenicity for Normal Rats

Twenty Sprague-Dawley rats were inoculated intraperitoneally with 0.5 ml. of a broth culture of the "yellow bacillus." Ten animals were given 10 mg. of cortisone twice each week. Two animals from the group injected with cortisone and 2 from the group not injected with cortisone were sacrificed at the end of the second, fourth, sixth, tenth, and twentieth weeks.

The gross findings in the group not injected with cortisone and sacrificed at the end of two weeks consisted of thickening of the omentum and small white nodules in the lungs and spleen which measured 1 to 2 mm. in diameter. The lesions were more numerous and larger in the animals sacrificed at the end of the fourth and sixth weeks. In the animals sacrificed at the end of the tenth and twentieth weeks, the size and number of the lesions had slightly diminished. Several of the animals had enlarged spleens without demonstrable gross lesions.

Microscopically, in the animals sacrificed at the end of two weeks, numerous small cellular granulomas were found in the liver, spleen (Plate III, figure 11), omentum, and abdominal lymph nodes; only a few granulomas were observed in the lungs. Unlike the lesions in hamsters, the splenic lesions in rats appeared to arise in Billroth's cords and not in the malpighian corpuscles. In the animals sacrificed at the end of four and six weeks, the lesions were larger than previously noted and showed a greater tendency to necrosis. At the tenth week, both cellular and necrotic lesions were observed in the lymph nodes and spleen. At the end of the twentieth week, the lesions had diminished slightly in size.

Acid-fast stains revealed the presence of a few organisms in the granulomatous lesions in animals sacrificed at the end of two weeks. A greater number of organisms were found in the lesions of the animals killed in the fourth, sixth, and tenth weeks but fewer were found in animals sacrificed during the twentieth week.

Pathogenicity for Rats Given Cortisone

In the group of animals treated with cortisone, the gross lesions were not as conspicuous either in size or in number as in the group not treated with cortisone. The microscopic lesions in the group treated with cortisone were similar in number, size, and cellular structure to those in the group not treated with cortisone. Acid-fast stains revealed approximately the same number of organisms in the lesions in both groups.

Some of the rats injected with cortisone showed subcutaneous nodules grosssly similar to the nodules seen in the hamsters given cortisone. Microscopically, they differed in that the lesions in rats contained moderate numbers of acid-fast organisms and were surrounded by a granulomatous tissue reaction.

Cultures of the lungs, liver, and spleen in all but 2 animals in the group not treated with cortisone were positive for the "yellow bacillus." Cultures of the same tissues in the group treated with cortisone were positive in all instances.

Pathogenicity for Normal Mice

Twenty-three Carworth Farms white mice (CFW) were inoculated intraperitoneally with 0.5 ml. of a broth culture of the "yellow bacillus." Of these, 10 were injected with 4 mg. of cortisone twice each week. Of the group not given cortisone, 2 animals died during the first week and one died during the second week. At the end of the fourth week, 2 animals were sacrificed. During the sixth week and again during the tenth and twentieth weeks, two animals were sacrificed.

The post-mortem findings in the animals not injected with cortisone which were observed during the first and second weeks consisted primarily of peritonitis and necrotic granulomas in the retroperitoneal fat and mesentery. Large numbers of atypical acid-fast organisms were demonstrated in the lesions. Those animals sacrificed during the fourth week showed small cellular granulomas (Plate III, figure 12) in the liver, spleen, and lymph nodes. Many of the macrophages were typical foam cells, which have been described in *Mycobacterium tuberculosis* (human) infections in mice (7). In the lesions of one animal, giant cells were

found. Many acid-fast organisms were present in these lesions. At the end of six weeks, the lesions were smaller and consisted of cellular granulomas in which no organisms could be demonstrated. The animals observed during the tenth and twentieth weeks showed small cellular granulomas with areas of necrosis in which small numbers of atypical acid-fast organisms could be found.

Pathogenicity for Mice Given Cortisone

Five of the cortisone-injected animals died during the second week. Many abscesses containing pyogenic bacteria were found in the organs and a few atypical acid-fast bacilli were observed. Death of these animals was due to a pyogenic infection. The 4 animals which died during the third week showed cellular granulomas with the presence of many atypical acid-fast organisms. In addition, pyogenic abscesses were seen. The same lesions were seen in one animal which died during the fourth week.

Cultures of the tissues from both groups were positive for the "yellow bacillus."

Pathogenicity for Rabbits

Ten rabbits were inoculated intravenously with 0.5 ml. of a broth culture of the "yellow bacillus." None of these animals received cortisone. Four animals were sacrificed during the fourth week, 3 during the ninth week, and 2 during the twentieth week. One animal died during the fourteenth week.

One of the animals sacrificed during the fourth week showed granulomatous lesions in the liver and lungs. Cultures of these tissues were positive for the "yellow bacillus." The other 3 animals showed no gross or microscopic lesions, and cultures of the tissues were negative for the "yellow bacillus." In one animal which was sacrificed during the ninth week, a single peribronchial granuloma in one lung was found. An occasional organism was demonstrated by acid-fast stain. The cultures of the tissues were negative for the "yellow bacillus." No lesions were found in the other 2 animals. Acid-fast organisms could not be demonstrated in the tissues, and the cultures of the tissues were negative. The animal which died during the fourteenth week had a confluent bronchopneumonia and a fibrinous pleurisy. No acid-fast organisms were demonstrated in the tissues; the cultures were negative for the "yellow bacillus." No lesions occurred in the 2 animals sacrificed at the end of the twentieth week, and cultures of the tissues for the "yellow bacillus" were negative.

Seven of the rabbits were skin tested at the end of four weeks, and those remaining were retested at the end of eight weeks. None showed significant reactions to the test substances.

Pathogenicity for Chickens

The chickens were of a mixed breed and were fed on Staley's Egg Atoms which contained no antimicrobial drugs. A group of 6 chicks, sixteen days of age, were inoculated intraperitoneally with 0.5 ml. of a broth culture of the "yellow bacillus." Skin tests with human and avian tuberculin and homologous skin test

material were performed at the end of one month. Two of 5 birds developed a 3-plus reaction to the avian tuberculin; reactions to human tuberculin and homologous skin-test material were not significant. The chicks grew rapidly and developed normally except for one which died three days after inoculation. Examination of the tissues from this animal was unsatisfactory due to tissue autolysis. Cultures of the tissues were positive for "yellow bacillus." The 5 other chicks were sacrificed after four months; no acid-fast organisms could be demonstrated in the tissues. Cultures of the tissues were negative for "yellow bacillus."

A group of 9 adult hens were inoculated intramuscularly with 0.5 ml. of a broth culture of the "yellow bacillus." They were skin tested prior to inoculation and were negative to avian tuberculin. Five of these birds were given 20 mg. of cortisone twice each week for the duration of the experiment. Tuberculin tests were performed at the end of two months. One bird in the group given cortisone developed a 3-plus reaction to human tuberculin and another a 4-plus reaction to both human and avian tuberculin. The other animals of this group did not show a significant reaction to the avian, human, or homologous skin test material. Of the 4 adult birds which were not injected with cortisone, one developed a 3-plus reaction to human and a 4-plus reaction to avian tuberculin. Another developed a 4-plus reaction to human, a 4-plus reaction to avian, and a 3-plus reaction to homologous skin-test material. The skin tests of the other 2 chickens were not significant. All the birds showed consistent weight gain, produced eggs regularly, and showed no signs of disease. They were sacrificed five months after inoculation. The tissues showed no evidence of disease grosssly or microscopically, and cultures of the tissues were sterile.

DISCUSSION

Culturally, the "yellow bacillus" does not resemble the human or bovine strains of *M. tuberculosis*; its cultural characteristics more closely resemble *Mycobacterium avium*, but it usually produces more pigment. In addition, the pathogenicity for various animal species clearly differentiates this organism from the human, bovine, and avian strains. Progressive disease does not occur in guinea pigs, although a large local lesion and occasional microscopic visceral lesions can be demonstrated in the animals sacrificed two or four weeks after inoculation. Following intraperitoneal injection, in animals given cortisone, granulomatous lesions appear in the lungs and progress for as long as eight to eleven weeks. These lesions also partially regress by the end of twenty weeks. The limited pathogenicity for guinea pigs sharply distinguishes this organism from the human and bovine tubercle bacilli. Intramuscular inoculation of the "yellow bacillus" in adult chickens and intraperitoneal inoculation in chicks fails to produce either gross or microscopic evidence of disease. Intravenous inoculation of rabbits with the "yellow bacillus" produces lesions in a few animals sacrificed early. In other animals sacrificed later, no significant lesions are found. As *M. avium* characteristically produces progressive disease in both birds and rabbits (8), a clear distinction between the avian organism and the "yellow bacillus" is demonstrated.

The present study demonstrates that Syrian golden hamsters are suitable

animals for the study of infection produced by the "yellow bacillus" as they consistently develop progressive disease when inoculated with large numbers of these organisms. A study is now in progress to determine the minimal infective dose. White mice are also highly susceptible to infection with this organism. The individual variation in the host-parasite relationship between different mice of the CFW strain is so great that this strain is not satisfactory for the study of this infection. The susceptibility to infection of hamsters and mice is in contrast to the resistance to infection demonstrated by white rats, guinea pigs, rabbits, and chickens.

Previous workers have repeatedly emphasized (9–12) that the lesions produced by *M. tuberculosis* infection in the hamster are much more apparent microscopically than grossly. A similar observation is strikingly demonstrated in this study. The extent of the lesions observed microscopically is usually much greater than that observed grossly, and it is not uncommon for an animal to show equivocal or no lesions grossly, but to show extensive disease microscopically. This is particularly true of animals given cortisone. Here the disparity is so great that grossly the animals given cortisone appear to have less disease than the animals not given cortisone, whereas microscopically it is apparent that the opposite situation exists.

The effects of cortisone in the dosages given on the host-parasite relationship is also apparent in these studies. The effects of large doses of cortisone were not studied. Cortisone was given to approximately one-half of all the animals used in the experiment except the rabbits. The animals that are relatively or absolutely resistant to the "yellow bacillus," namely, rats and chickens, show no particular alteration in the disease process when given cortisone. Guinea pigs, although relatively resistant to this disease, show more evidence of dissemination of the infection when given cortisone. The effect on mice is difficult to evaluate, as many of these animals die of intercurrent infection enhanced by cortisone. In hamsters, which are highly susceptible to the "yellow bacillus," the effects of cortisone administration are clearly demonstrated. The data given in table 1 and emphasized in figure 1 show that the group of animals given cortisone died of the disease more rapidly than the group not given cortisone. Other effects consist of an increase in the amount of necrosis and an increase in the number of organisms demonstrable in the lesions. Grossly, the lesions tend to be smaller and less conspicuous in the animals given cortisone than in the animals not given cortisone. Microscopically, it is apparent that the difference between the two groups is due to the presence of fewer cellular elements in the lesions, even though there is greater destruction of tissue in the group given cortisone.

SUMMARY

A brief review of the cultural characteristics of an atypical acid-fast organism ("yellow bacillus") which produces disease in humans is presented. The pathogenicity of this organism for several animal species varies. Large doses of this organism fail to produce progressive disease in the guinea pig, chicken, and rabbit, produce variable amounts of disease in the mouse and the rat, and consistently produce progressive fatal disease in the Syrian golden hamster. Corti-

sone administration increases the severity of the disease in the hamster, but has little or no effect in the dosages used on guinea pigs, rabbits, and chickens.

SUMARIO

Las Características Culturales y Patogenicidad para los Animales de un Microbio Acidorresistente Atípico que Provoca Enfermedad Humana

Ofrécese una breve reseña de las características de cultivo de un microbio ácidorresistente atípico ("bacilo amarillo") que produce enfermedad en seres humanos. La patogenicidad de este microorganismo para varias especies animales varía. Grandes dosis del mismo no provocan afección evolutiva en el cobayo, el pollo o el conejo, producen enfermedad más o menos acentuada en el ratón y la rata y producen constantemente enfermedad letal evolutiva en el hamster dorado sirio. La administración de cortisona acrecienta la gravedad de la enfermedad en el último animal, pero a las dosis usadas, ejerce poco o ningún efecto en los cobayos, conejos y pollos.

RESUME

Caractéristiques culturelles et pathogénicité pour l'animal d'un microorganisme acido-résistant atypique produisant l'affection humaine

Présentation d'un bref exposé sur les caractéristiques structurales d'un bacille acido-résistant ("bacille jaune") produisant la tuberculose chez l'homme. La pathogénicité de cet organisme varie chez diverses espèces animales. L'administration de l'organisme à doses fortes ne parvient pas à produire l'affection évolutive chez le cobaye, le poulet et le lapin; elle la provoque à un certain degré chez la souris et le rat; chez le hamster doré de Syrie, elle détermine constamment la forme évolutive fatale. L'administration de cortisone accroît la sévérité de l'affection chez le hamster, mais elle n'exerce que peu ou pas d'action avec les doses utilisées chez les cobayes, lapins et poulets.

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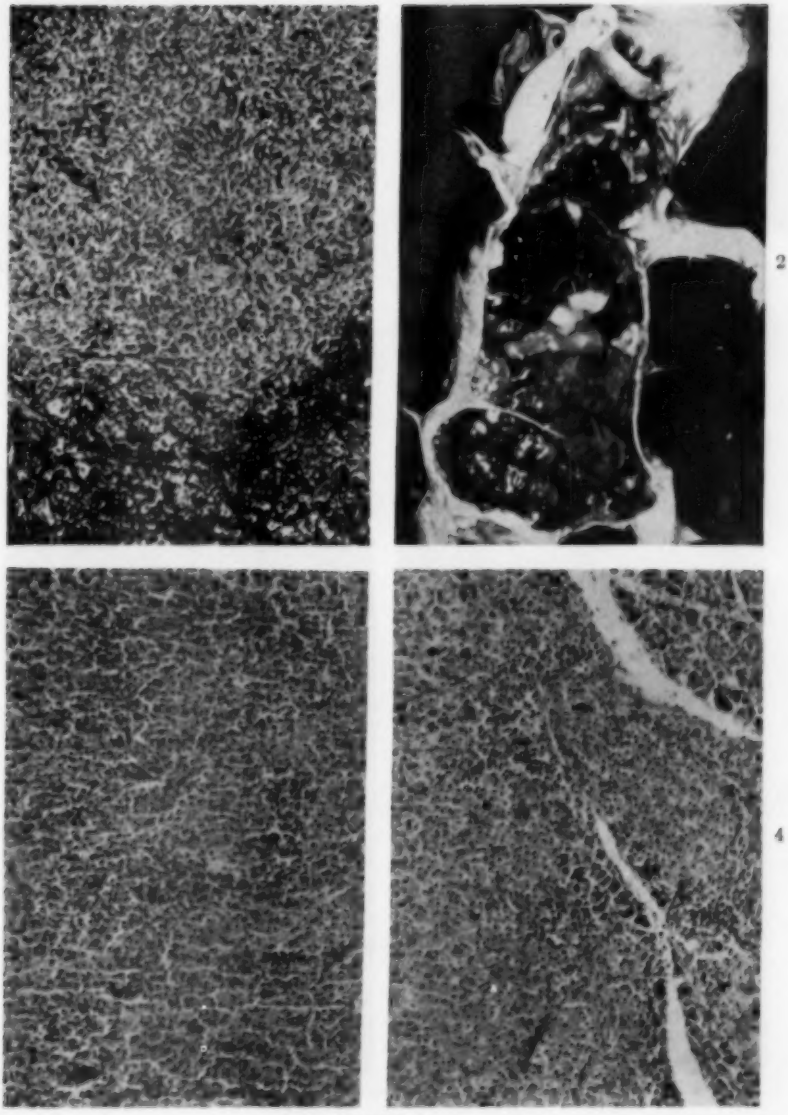


PLATE I

FIG. 1. Guinea pig. No cortisone. Lymph node showing a granuloma. Hematoxylin and eosin (×110)

FIG. 2. Hamster. No cortisone. White nodular masses in liver, mesentery, and on the surface of the spleen.

FIG. 3. Hamster. No cortisone. Lymph node completely replaced by a granuloma composed of macrophages. Hematoxylin and eosin (×110)

FIG. 4. Hamster. No cortisone. Pancreas partly replaced by granuloma; only a few pancreatic acini remain in the center and the right upper corner of the field. Hematoxylin and eosin (×110)

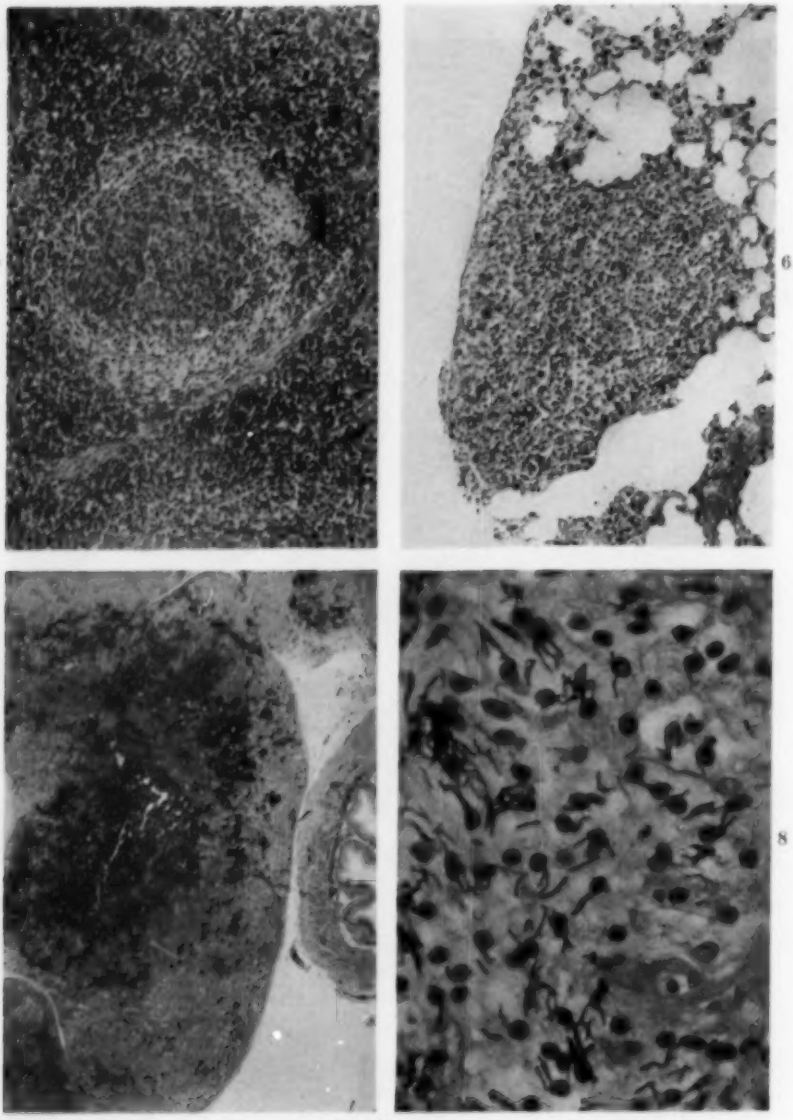


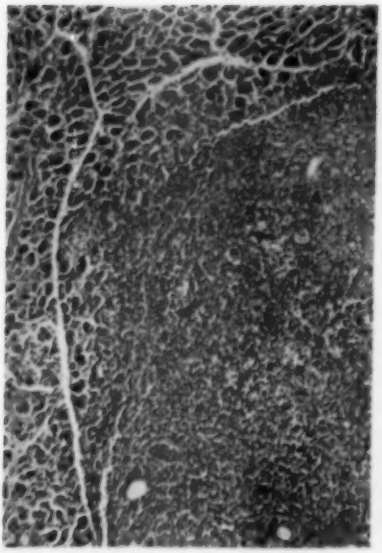
PLATE II

FIG. 5. Hamster. No cortisone. Spleen showing a granuloma occupying the center of a malpighian corpuscle. Hematoxylin and eosin (×110)

FIG. 6. Hamster. No cortisone. Lung showing a subpleural granuloma. Hematoxylin and eosin (×110)

FIG. 7. Hamster. No cortisone. Lymph node showing a large partly calcified granuloma. The calcified areas stain more deeply. A portion of seminal vesicles can be seen at the right. Hematoxylin and eosin (×14)

FIG. 8. Hamster. No cortisone. Acid-fast stain of lung lesion showing numerous organisms. Fite stain (×685)



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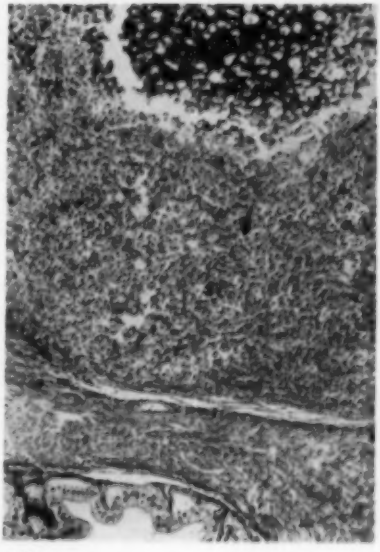

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PLATE III

FIG. 9. Hamster. Cortisone. Necrotic lesion in the pancreas. Only nuclear fragments are visible. Hematoxylin and eosin (×125)

FIG. 10. Hamster. Cortisone. Acid-fast stain of a lymph node showing the many organisms present. Fite stain (×685)

FIG. 11. Rat. No cortisone. Spleen showing small granulomas arising in Billroth's cords. Hematoxylin and eosin (×110)

FIG. 12. Mouse. No cortisone. A granulomatous lesion with central necrosis adjacent to the genital tract. A portion of the seminal vesicles can be seen at the bottom of the figure. Hematoxylin and eosin (×62)

OBSERVATIONS ON A PLEOMORPHIC MYCOBACTERIUM ISOLATED FROM MOUSE BRAINS[1, 2]

I. Cultural and Morphologic Characteristics

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INTRODUCTION

The mycobacterium described in this report was first isolated from a pool of mouse brains. This was prepared from animals which had been inoculated intracerebrally with mouse material containing an unidentified agent associated with the occurrence of acute myocarditis and pulmonary arteritis (1).

To investigate the possible relationship of this agent to the pleuropneumonia group, the mouse brain suspension and several other infective materials (chorioallantoic membranes and yolk sacs) were inoculated into liquid medium (PPLO broth described below). From the third blind passage on this medium, plates were made after addition of 1.6 per cent agar. After five days fine colonies were observed which did not resemble colonies of pleuropneumonia-like organisms. Gram stains revealed slender, irregularly gram-positive rods. Smears from the fluid media showed similar bacillary forms with deeply staining granules. The granules and the grouping suggested mycobacteria, and it was discovered at this time that the microorganisms were indeed acid-fast.

The egg materials cultured in the same way at the same time did not yield bacteria. The acid-fast microorganism, designated as *Mycobacterium X*, was reisolated from another sample of the same brain pool which had been sealed and stored for four months at −70°C. This would indicate that the mouse brain suspension was actually the source of this mycobacterium and that it had not been accidentally introduced during the cultural procedures. The possibility that the stock mice might have been harboring this mycobacterium was considered. Twenty normal mice from four breeding rooms were sacrificed. Brain, spleen, and liver were ground separately in mortars and samples were inoculated into PPLO broth, Tween®-albumin medium and oleic-acid–albumin agar. These cultures were incubated for six weeks. In no instance could a mycobacterium be recovered.

It should be noted that this mycobacterium plays no part in the pathogenesis of the myocardial and pulmonary lesions which have been found to be associated with rickettsia-like bodies in polymorphonuclear leukocytes (1). In no instance have these mycobacteria been demonstrable in such lesions. As will be shown in an accompanying paper (Paper II), the pathologic changes following experimental infection with *Mycobacterium X* are of an entirely different character.

[1] From the Department of Bacteriology and Immunology, Harvard Medical School; and the Massachusetts Department of Public Health, Boston, Massachusetts.

[2] This work was supported by a grant from Geigy Pharmaceuticals, Inc., New York New York.

Because of its unusual source, further studies seemed indicated. The present paper is concerned with the morphologic and cultural characteristics of this strain, and an accompanying paper (Paper II) describes its pathogenetic effects in laboratory animals. In an effort at identification, comparative studies with other known strains were carried out.

MATERIALS AND METHODS

The basal medium of Dubos and Middlebrook (2) was used in the first three preparations. The figures express the final concentration for all media.

Tween®-albumin medium	basal medium	
	Tween® 80	0.05%
	bovine albumin, fraction V	0.5%
Liquid oleic-acid medium	basal medium	
	oleic acid	0.006%
	bovine albumin, fraction V	0.5%
	glucose	0.5%
Oleic-acid agar:	liquid oleic acid medium containing Bacto agar (Difco)	1.5%
Löwenstein's medium: Jensen's (3) modification		
Tryptic digest broth:	15% ground beef heart in water, digested with trypsin, filtered through paper, neutralized.	
	NaCl	0.5%
	glucose	0.1%
Blood agar:	tryptic digest broth	
	Bacto agar	2%
	horse blood defibrinated	5%
Sabouraud's medium:	Bacto peptone	1%
	Bacto agar	1.5%
	glucose	4%
Thioglycollate medium:	Baltimore Biological Laboratory heart infusion peptone broth	
PPLO medium:	Seitz-filtered horse serum	20%
	glucose	0.5%

Animals: Swiss white mice originally obtained from V. Schwentker were bred in the animal house of Harvard Medical School. Guinea pigs, rabbits, and chickens were obtained from outside sources.

Tissue cultures: Cultures of macrophages, which were collected from peritoneal exudates of guinea pigs or mice five days after the injection of a chemotactic agent, were prepared and infected following the slide technique of Suter (4).

Slide cultures of mycobacteria under formvar without macrophages: Suspensions of mycobacteria were the same as those prepared for the infection of the monocyte cultures. The bacteria were allowed to settle down on coverslips. After sedimentation the coverslips were removed, covered with a thin film of formvar, and placed into a small vial containing 0.5 ml. of medium (Tween®-albumin medium, liquid oleic acid medium, or basal medium with 0.5 per cent glucose and 40 per cent guinea pig serum). Slides were removed at daily intervals, fixed in methyl alcohol, and stained according to the method of Ziehl-Neelsen.

Stock cultures for comparative studies:

Bovine strain "Vallée," obtained from Dr. H. Bloch, Public Health Research Institute of the City of New York, New York

Avian virulent strain "Sheard," obtained from Dr. E. Wolinsky, Trudeau Laboratory, Saranac Lake, New York
Avian avirulent strain "A 1," obtained from the same source
Vole bacillus "OV 183," obtained from the same source

All strains were maintained in this laboratory in Tween®-albumin medium, on oleic-acid agar, and on Löwenstein-Jensen medium.

RESULTS

The comparative studies with stock strains are summarized in table 1 and demonstrate the similarity of *Mycobacterium X* to the avian strains.

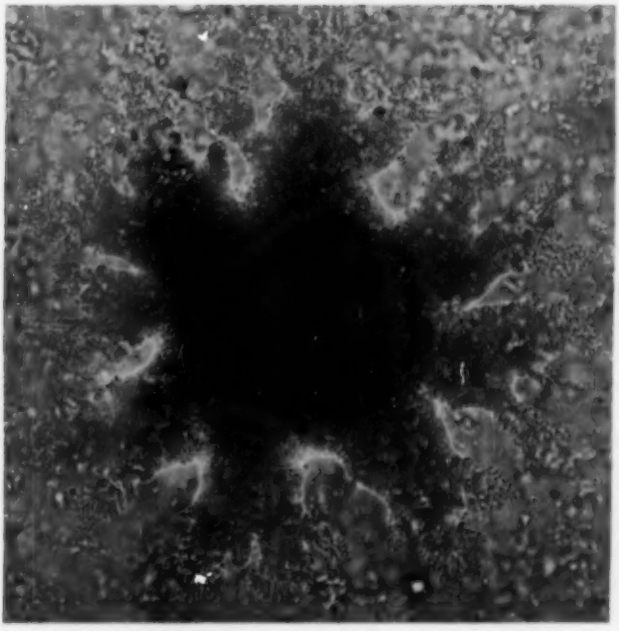
Growth on Various Media

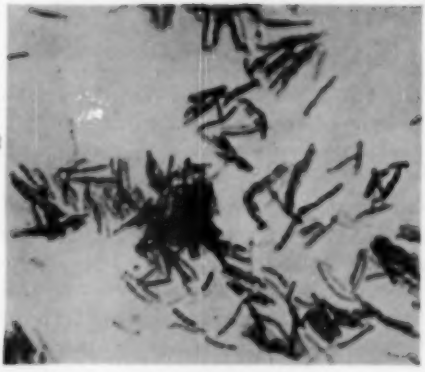
Oleic-acid agar medium: Small inocula on oleic-acid agar medium yielded isolated colonies; growth became visible to the naked eye after approximately five to seven days. By low-power observations the colonies showed a characteristic appearance which became more distinctive after fourteen days. There was a slightly raised yellowish opaque center which gradually flattened toward the periphery where it was translucent: the edge was fimbriated and the colony had a stellate appearance (figure 1). In general, the size of the colony did not exceed 1 mm. in diameter. However, a second colonial form was noted which was spherical and opaque. This type occurred if *Mycobacterium X* was isolated directly from animal organs and was in contact with residual tissue. Although this variation in colony morphology may depend in part upon the medium or the presence of tissue in the inoculum, nevertheless both types of colony may be found on the same plate in different proportions. The stellate colony resembles that of the virulent avian strain "Sheard." The compact colony is similar to the avirulent avian strain "A 1." The colonies produced by the "Vallée" and vole strains are of an entirely different character.

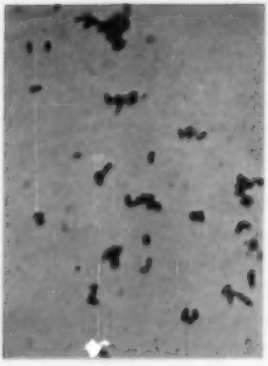
TABLE 1

CULTURAL CHARACTERISTICS OF MYCOBACTERIA

Strain	Oleic-Acid Agar	Löwenstein	Tween®-Albumin Liquid	Oleic-Acid Liquid	Morphology
Mycobacterium X	Moist Compact colonies Stellate colonies	Smooth Moist Homogeneous	Netlike sediment	Sediment dispersed on shaking	Highly pleomorphic Branching Free granules
Sheard	Moist Stellate colonies but smaller	As above	As above	As above	Pleomorphic Branching Free granules
A1	Moist Compact colonies Larger than *Mycobacterium X*	Same colonies, but more abundant growth	Sediment more compact	As above	As above
Vallée	Dry, colonies with cord formation	Dry, rough, heaped up	Compact sediment	Flocculent	Only bacillary forms
Vole	Dry, small, irregular colonies	Dry, rough but scant growth	Compact sediment	Flocculent	Bacillary forms with hooked ends







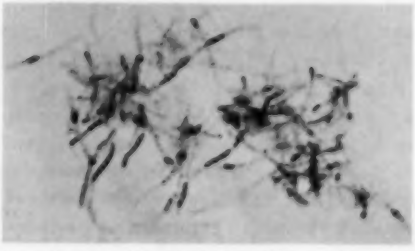


FIG. 1. Fourteen-day colony of *Mycobacterium X* on oleic-acid agar. Low power

FIG. 2. *Mycobacterium X*. Six-day growth in PPLO medium. Beaded bacillary forms. Ziehl-Neelsen (×1500)

FIG. 3. *Mycobacterium X*. Ten-day Tween®-albumin culture. Granular forms. Ziel-Neelsen (×1500)

FIG. 4. *Mycobacterium X*. Five-day microcolony on coverslip covered with formvar, growth in 40 per cent rabbit serum and 60 per cent basic medium. Ziehl-Neelsen (×1040)

Tween®-albumin medium and liquid oleic-acid medium: Growth in these fluid media became visible after three to seven days and increased up to fourteen days. If the culture tube remained undisturbed, a delicate netlike structure was formed by the sedimented bacilli and the supernatant fluid was clear. The medium showed diffuse turbidity when the tube was shaken. No clumping occurred even in media without Tween® 80. When the amounts of Tween® 80 in these media were increased to 0.6 per cent, *Mycobacterium X* grew more abundantly in the higher concentrations. All of these characteristics are like those of the avian strains and unlike those observed with the "Vallée" and vole strains.

Jensen-Lõwenstein medium: On Jensen-Lõwenstein medium the colonies first became visible after one to two weeks' incubation. At three weeks they were flat, moist, smooth, and yellowish-white. They could be taken up in saline to form a homogenous suspension. They were not chromogenic, even after prolonged incubation. Growth on this medium never became as abundant as the growth of the "Vallée" strain. In this respect and in the colony morphology, *Mycobacterium X* again resembles the avian strains.

Growth on *blood agar*, *plain agar*, and *Sabouraud's medium* was very slow, and the colonies were small and shiny. In *tryptic digest broth* growth became visible only after six weeks' cultivation. In *thioglycollate broth* slight turbidity just underneath the surface could be observed after six weeks.

Morphology

In the primary isolation in PPLO medium the organisms were slender bacilli. When stained by the Ziehl-Neelsen technique, the organism was strongly acid-fast, resisting decoloration with 3 per cent hydrochloric-acid-alcohol for more than four hours.

A characteristic feature of the microorganism was its granularity, which was sometimes so pronounced that the bacillus appeared as a chain of cocci (figure 2). Granules attained a diameter of 2 μ. This occurred more often in Tween®-albumin cultures than in other media. Indeed, the individual granules often became dissociated from the bacilli and were found free in the medium (figure 3). The formation of the free granules was dependent on the medium in which the organisms were grown. Free granules occurred very readily in media containing Tween® 80 or oleic acid, but were not found in the medium with serum alone.

In addition to these acid-fast granules, there were granules which stained with methylene blue, hematoxylin, or Giemsa's stain. These basophilic granules have been interpreted as being nuclear in character (5) or mitochondria (6). Their location varied. They were polar or distributed at irregular intervals along the length of the bacillus. In some cultures the basophilic substance had the shape of rods (figure 4). When grown on slides, as described above, long filamentous forms appeared which were frequently branched. These pleomorphic characters were similar to those seen with the avian strains, but even more pronounced. The hooked end, which is a characteristic feature of the vole bacillus, was not observed with *Mycobacterium X*.

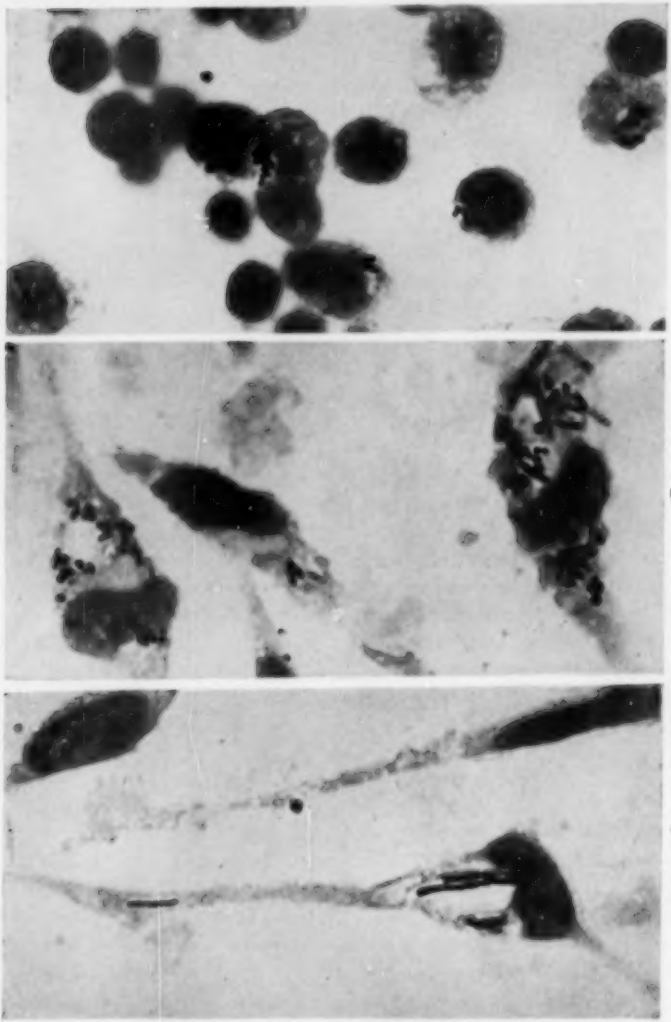


FIG. 5. Culture of mouse monocytes immediately after phagocytosis of acid-fast granules of *Mycobacterium X*, obtained from ten-day Tween®-albumin culture. Carbolfuchsin and hematoxylin (×1500)

FIG. 6. Culture of mouse monocytes three days after infection with granular forms of *Mycobacterium X*. Stages of the development of granular forms into bacilli. Carbolfuchsin and hematoxylin (×1500)

FIG. 7. Mouse monocyte culture, five days after infection with granular forms of *Mycobacterium X*. The granules have developed into long bacilli. Carbolfuchsin and hematoxylin (×1500)

Filtration Experiments

Because of the small size of the granules and the controversy regarding the filterability of tubercle bacilli, it was of interest to ascertain the behavior of *Mycobacterium X* in this respect. Both PPLO and Tween®-albumin cultures failed to pass through Seitz filters. Mice inoculated with filtrates developed no lesions.

Tissue Culture Experiments

The intracellular behavior of *Mycobacterium X* was also studied in cultures of macrophages as described above. The macrophages were infected with bacilli grown in Tween®-albumin medium for seven to ten days. Single coverslips were fixed in methyl alcohol, stained with carbolfuchsin, and counterstained with hematoxylin. Counts of intracellular acid-fast granules or bacilli in these tissue cultures were made. As the Tween® cultures used for infection consisted almost exclusively of granular forms as described above, the macrophages at the beginning of cultivation contained only acid-fast granules (figure 5). No significant increase of the number of these forms could be observed throughout the period of cultivation. Intracellular bacillary forms appeared and increased in number during the seven days of cultivation (figures 6 and 7). In occasional cultures, when a few bacilli remained unphagocytized, luxuriant extracellular growth occurred in the form of long filaments. The intracellular forms had not multiplied to nearly the same degree, suggesting that the intracellular environment was a less favorable one for *Mycobacterium X*.

It was consistently observed that bacillary forms appeared earlier and in greater number in macrophages from mice than in those from guinea pigs. In the latter, increased numbers of bacillary forms were observed only after seven days of cultivation.

Animal Experiments

Guinea pigs and chickens were inoculated with a seven- to ten-day Tween®-albumin culture intravenously and intraperitoneally, and a rabbit was inoculated intravenously. Mice were inoculated by the same routes and, in addition, subcutaneously and intracerebrally. None of these animals showed any evidence of disease during life; microscopic lesions were minimal in guinea pigs, chickens, and in the rabbit. Mice showed more intensive and interesting lesions which will be described in the following paper. Guinea pigs injected with *Mycobacterium X* did not develop hypersensitivity to tuberculin derived from tubercle bacilli of human origin.

Discussion

Although the recovery of a new strain of mycobacterium from mouse tissue in the absence of obvious disease would in itself be of little interest, detailed study of this particular organism has disclosed certain features which merit discussion.

Because of its low virulence for laboratory animals, the classification encounters

considerable difficulty. *Mycobacterium X* does not have the characteristics of a saprophyte; it does not produce pigment, it grows only slightly on plain agar after prolonged incubation, grows slowly on media designed for mycobacteria, and possesses the property of invasiveness for the mouse. It can be differentiated readily from the human and bovine tubercle bacilli by the following criteria: greater pleomorphism of the new strain, colony morphology, easily dispersible growth in liquid media without the use of Tween® 80, and failure of guinea pigs to develop hypersensitivity to tuberculin derived from tubercle bacilli of human origin.

Mycobacterium X differs from the vole bacillus in its microscopic and colony morphology and its easily dispersed growth in liquid media without the use of Tween® 80.

On the other hand, there is no sharp difference between it and the virulent or avirulent avian bacilli, which also show branching under certain conditions and a tendency to form free granules. The growth in Tween®-albumin medium and liquid oleic-acid medium is also indistinguishable from that of the two avian strains. *Mycobacterium X* produces two types of colonies on oleic-acid agar: one is almost identical with that formed by the virulent avian tubercle bacillus "Sheard" and the other with that formed by the avirulent avian strain "A 1." Two distinct types of colonies with the attenuated bovine strain BCG were described by Suter and Dubos (7). *Mycobacterium X* may represent a similar situation in the avian group. This conception is consistent with its limited pathogenicity for animals.

An interesting feature of *Mycobacterium X* is the production of free acid-fast granules. There are two opposing views about these granules: (*1*) that they are stages in the disintegration of the bacilli, or (*2*) that they are stages in a developmental cycle and capable of growth into bacillary forms. A good discussion will be found in the book by Rich (8) in which arguments on both sides of this question are adduced. With avian tubercle bacilli, it has recently been demonstrated by phase-contrast studies (9) and electronmicroscopy (10) that the granules are part of the developmental cycle and not a degradation product. The present observations support this interpretation.

Intracellular multiplication in guinea pig monocytes was consistently slower than in mouse monocytes. This may indicate that the resistance of guinea pigs to *Mycobacterium X* is a native or innate resistance and that it may be related to the relatively greater ability of the macrophages to limit the intracellular multiplication of *Mycobacterium X*.

SUMMARY

A pleomorphic, acid-fast mycobacterium was isolated from a suspension of mouse brains. Morphologic and cultural characteristics were studied and compared with those of other known strains of mycobacteria. The new strain does not differ in any significant respect from the avian group; its pathogenicity is of a low order. Free granules produced are thought to be viable units and able to develop into rods.

SUMARIO

Observaciones sobre una Micobacteria Pleomorfa Aislada de Cerebros de Ratones: I. Características Culturales y Morfológicas

Una micobacteria ácidorresistente pleomorfa fué aislada de una suspensión de cerebros de ratones. Se estudiaron las características morfológicas y culturales, comparándolas con las de otras cepas conocidas de micobacterias. La nueva cepa no discrepa en ningún sentido importante del grupo aviario; su patogenicidad es baja. Los gránulos libres producidos parecen ser unidades viables, capaces de convertirse en bastoncillos.

RESUME

Remarques sur une mycobactérie pléomorphique isolée du cerveau de souris. I. Caractères culturaux et morphologiques.

Une mycobactérie acido-résistante a été isolée à partir d'une suspension de cerveau de souris. Les caractères culturaux et morphologiques du germe ont été étudiés et comparés avec ceux d'autres souches connues de mycobacterium. La nouvelle souche ne diffère pas notablement du groupe aviaire; sa pathogénicité est réduite. Les granules libres produits sont considérés comme des éléments viables aptes à se transformer en bâtonnets.

Acknowledgment

For helpful advice in the course of this work, the writers are indebted to Dr. Clarke Gray (Leonard Wood Memorial, Boston, Massachusetts) and to Dr. Emanuel Suter of the Department of Bacteriology and Immunology, Harvard Medical School.

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OBSERVATIONS ON A PLEOMORPHIC MYCOBACTERIUM ISOLATED FROM MOUSE BRAINS[1, 2]

II. Pathology and Comparison of Lesions with Those Produced by Certain Other Strains of Mycobacteria

A. M. PAPPENHEIMER, J. DANIELS, AND W. A. VISCHER

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INTRODUCTION

In an accompanying paper (Paper I), the morphology and cultural features of a mycobacterial strain originally isolated from mouse brain suspension have been described (1). In the hope of defining this microorganism more closely, a study has been made of the pathologic changes induced by experimental infection in mice and in other laboratory animals. The lesions thus produced have been compared with those attending infection with certain other mycobacteria. It was not within the scope of this work to make an exhaustive comparative study of mycobacterial infections in mice. This would have involved careful standardization of dosage, the selection of susceptible or resistant strains of inbred mice, and other factors which have been ignored in the present work. It is the purpose rather to present briefly the lesions found after inoculation of *Mycobacterium X* by various routes and to show how these resemble or differ from those caused by certain other representative strains of nonsaprophytic mycobacteria.

OBSERVATIONS

Pathology

The study of the pathologic changes induced by *Mycobacterium X* consisted of the examination of 93 mice. Of these, 22 were inoculated intravenously; 21, intraperitoneally; 5, subcutaneously; 5, intranasally; 43, intracerebrally. Included are control animals injected with heat-killed cultures and filtrates.

Tissues were fixed in Bouin's or Zenker's fluid. The classical Ziehl-Neelsen method and hematoxylin counterstain gave satisfactory preparations.

None of the infected mice showed signs of illness during life, and it was not possible to detect gross lesions at autopsy.

Tissue reaction: The tissue reaction to this microorganism was a simple one. It consisted of minute tubercles, composed of a central group of large pale epithelioid cells, with vesicular nuclei and abundant, eosinophilic cytoplasm, surrounded by a variable number of small lymphocytes. The epithelioid cells showed no radial disposition. They had not undergone caseous necrosis, nor had they fused to form giant cells of the Langhans' type. In the preparations thus far examined, the tubercles had not undergone fibrous replacement.

[1] From the Department of Bacteriology and Immunology, Harvard Medical School; and the Massachusetts Department of Public Health, Boston, Massachusetts.

[2] This work was supported by a grant from Geigy Pharmaceuticals, Inc., New York, New York.

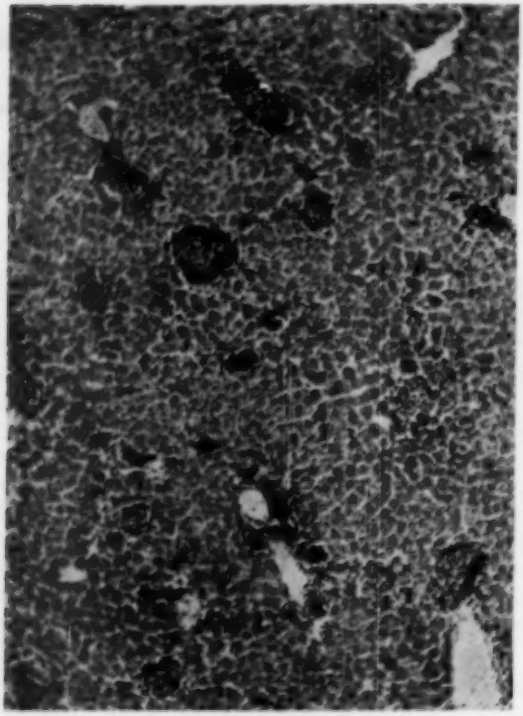

FIG. 1. *Mouse 6348.* Killed 21 days after intravenous injection of *Mycobacterium X*. Miliary tubercles in liver. Ziehl-Neelsen (×140)

Regardless of the route of inoculation, whether intravenous, intraperitoneal, subcutaneous, or even intracerebral, tubercles of this type were found in the *liver* in greater or lesser number (figure 1). This organ, indeed, appeared to be a site of predilection. After intraperitoneal inoculation, tubercles were found also in mesenteric and retroperitoneal lymph nodes. More interesting was the occurrence of a more diffuse type of reaction in the intra-abdominal adipose tissue. Isolated tubercles were also occasionally present in mediastinal lymph nodes and rarely in axillary lymph nodes.

The *spleen* was rarely affected but, occasionally, small groups of pale, swollen reticular cells were seen in the pulp. The *lungs* also were surprisingly resistant to this microorganism, even after intranasal infection. Groups of large mononuclear cells with a few lymphocytes, in which acid-fast bacilli could be demonstrated, were encountered in only a few instances. In several instances, tubercles were found in the mediastinal lymph nodes without obvious involvement of the lungs.

Acid-fast bacilli: Acid-fast bacteria were readily demonstrated in the lesions with the standard Ziehl-Neelsen stain (figure 2), but they were not usually very numerous and never formed compact colonies such as were found with the

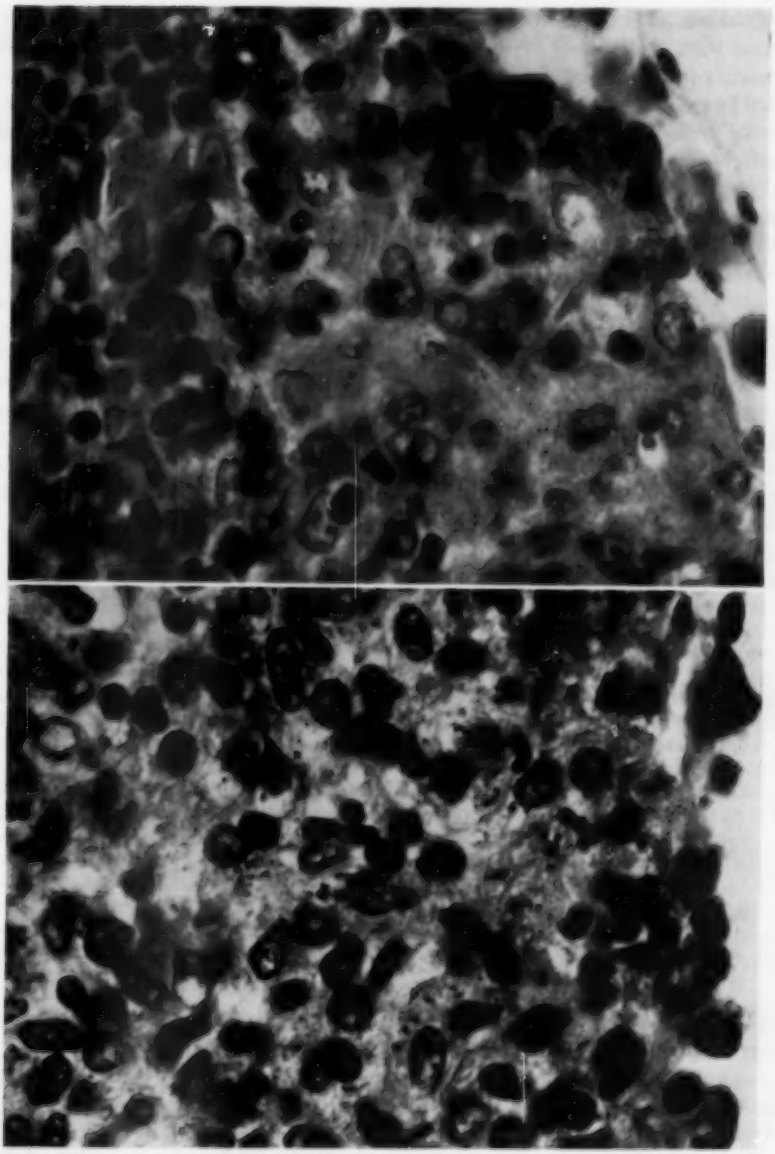

FIG. 2. *Mouse 6246.* (Top). Killed 35 days after intraperitoneal injection of *Mycobacterium X*. Bacillary forms in swollen reticular cells of mediastinal lymph nodes. Ziehl-Neelsen (×1500)

FIG. 3. *Mouse 6344.* (Bottom). Killed 16 days after intraperitoneal injection of *Mycobacterium X*. Granulomatous tissue in intraperitoneal fat, containing only granular acid-fast forms. Ziehl-Neelsen (×1500)

Sheard strain of virulent mycobacterium. Both granules and beaded bacillary forms were seen, often in the same lesions, but one or the other form might greatly predominate and, in some preparations, granules alone were seen (figure 3). The granules appeared as minute solid dots or as larger spherical or oblong bodies with a clear center. In the bacillary forms, basophilic granules staining with hematoxylin occurred along the course of the rods. In Giemsa-stained preparations, careful study disclosed dark blue granules in the cytoplasm of epithelioid cells; the bacilli and larger granules were unstained.

It was interesting that the presence of the bacilli within the cytoplasm of the mononuclear cells did not lead to any histologic evidence of degeneration. The nuclei of these phagocytic cells were well stained with normal chromatin structure.

Neurologic lesions: The lesions following intracerebral injection deserve special description, inasmuch as there are few previous studies with other mycobacterial strains in the literature. In spite of the rather severe character of the neurologic lesions, it has not been possible to detect any signs of meningeal irritation in the living mice. They have not shown hyper-excitability, convulsions, head retraction, or other signs customarily associated with inflammatory changes in the brain or meninges.

Examination was made of 29[2] mice sacrificed at intervals ranging from three to thirty-four days after intracerebral inoculation of 0.03 ml. of culture. The following brief descriptions illustrate the changes noted at different periods:

> *Mouse 6401:* Inoculated intracerebrally at three weeks of age with ten-day culture on PPLO medium; killed three days later. Over both *cerebrum* and *cerebellum*, there were discrete plaques of meningeal thickening, composed of epithelioid cells and rounded large mononuclears. These contained large numbers of long, acid-fast bacilli. There was no invasion of the underlying brain substance.
>
> *Mouse 6428:* Sacrificed eight days after intracerebral inoculation. The meninges showed no inflammatory reaction, such as was found in *Mouse 6401*, and contained no acid-fast bacilli. It was most interesting to note that there were many necrotic Purkinje cells and medullary ganglion cells which had lost their nuclei, and stained bright red with the fuchsin. Upon the surface of these dead cells or scattered through the neighboring tissue were acid-fast granules which were usually small but were occasionally larger with clear center; they were rarely short, plump bacillary forms. These bodies which are identical with the granular forms seen in cultures and in other lesions were restricted to the proximity of the necrotic cells. They had excited no inflammatory reaction.

That these bodies were extruded from the dead cells and represented, not mycobacteria, but degenerative cell debris, is unlikely for two reasons: they were morphologically indistinguishable from the granular forms of the bacilli, as seen in cultures; and they were only exceptionally found, although necrosis of Purkinje cells is present in all of the preparations of inoculated brains. As will be pointed out later, similar granules were found about the Purkinje cells and medullary ganglion cells in a mouse inoculated intracerebrally with the vole bacillus and killed on the seventh day after inoculation.

[2] Mice inoculated with filtrates or killed bacilli were not included.

While there is little doubt that these structures represent granular forms of the mycobacteria, their occurrence in these situations poses several as yet unanswerable questions. As there was no extension of the meningeal lesions to the deeper tissues, and the granular bodies were not found within microglial or other phagocytic cells, the manner in which they arrived at the Purkinje cell level is unexplained.

That they are implicated in the destruction of the ganglion cells is very improbable, for necrotic cells were so frequently unassociated with their presence. And yet the granules are unquestionably linked in some obscure way with these necrotic cells. They often lay in small indentations on their surface and, as has been stated, they were restricted in their geographic distribution to the neighborhood of the dead cells.

Meningitis, ventriculitis, and involvement of the choroid plexus were found as late as thirty-nine days after inoculation, and the bacilli persisted in the lesions. In some of the mice, tubercles were found in the liver, showing that dissemination had occurred.

Comparison of Lesions with Those Produced by Other Strains of Mycobacteria

In attempting to identify this strain of mycobacterium, it seemed desirable to compare its pathogenetic effects with those produced by other strains of acid-fast organisms.

There were available for this purpose a bovine strain "Vallée," obtained from Dr. Hubert Bloch, Public Health Research Institute of the City of New York; "Sheard" virulent avian strain isolated from a chicken in 1944 by Dr. W. Feldman and obtained from Dr. E. Wolinsky, Trudeau Laboratory, Saranac Lake, New York; "A 1" avirulent avian strain, obtained from Dr. E. Wolinsky, Trudeau Laboratory, Saranac Lake, New York; "OV 183" vole bacillus, isolated by Dr. A. Q. Wells and obtained from Dr. E. Wolinsky, Saranac Lake, New York. An opportunity was also afforded to study livers and spleens of dba mice infected with murine leprosy bacilli.[4] The lesions caused in mice by virulent and avirulent human strains have been so thoroughly studied that it seemed unnecessary to include them (2-4).

Lesions caused by the Vallée strain: Of the strains tested, the Vallée strain proved to be the only one which caused emaciation and death of the mice. The lesions in 6 animals were studied: 2 were inoculated intracerebrally, 2 intravenously, 2 intraperitoneally. The mice appeared to be relatively resistant to infection by this last route.

After intravenous injection of 0.2 ml. of an eleven-day culture on Tween®-albumin medium, intense lesions were found in myocardium, lungs, liver, spleen, and kidneys.

In the *heart*, after thirty-five and forty-two days, there were focal collections of large monocytes, lymphocytes, and some polymorphonuclears between the muscle fibers, usually in the vicinity of blood vessels (figure 4). Acid-fast bacilli were very abundant within the mononuclears. There was no necrosis. In one of

[4] The writers are greatly indebted to Dr. Gladys Hobby of Chas. Pfizer & Co., Inc. for this material.

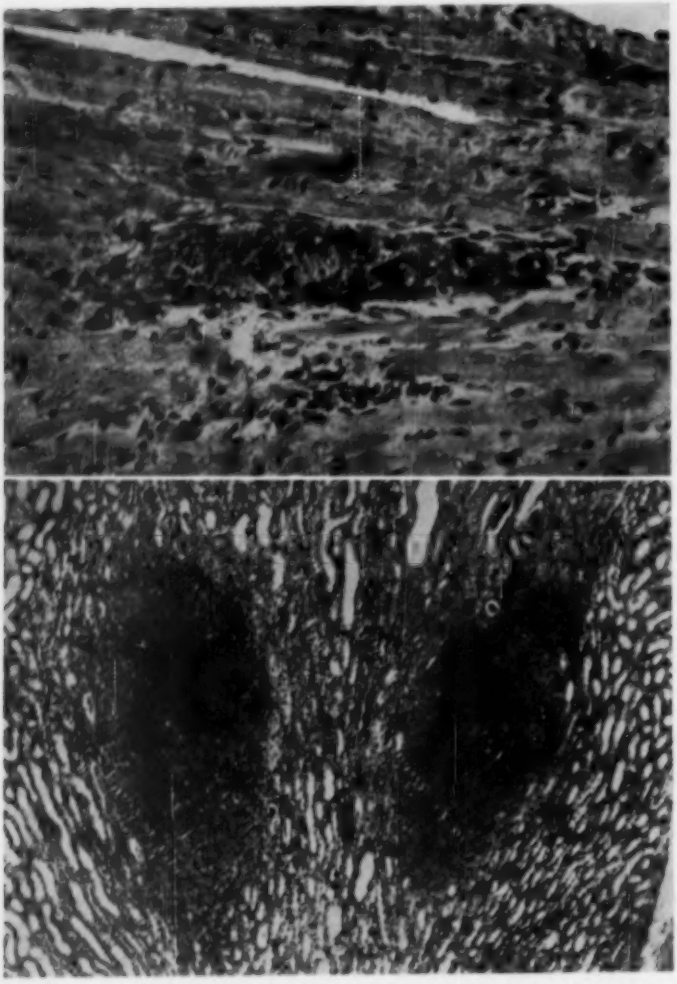

FIG. 4. *Mouse 6640.* (Top). Vallée strain inoculated intravenously, killed after 35 days. Myocardium showing collections of macrophages stuffed with acid-fast bacilli and surrounded by lymphocytes. Ziehl-Neelsen (×430).

FIG. 5. *Mouse 6641.* (Bottom). Vallée strain, inoculated intravenously, killed after 42 days. Large areas of necrosis containing many acid-fast bacilli in renal pyramid. Ziehl-Neelsen (×110)

the mice (*Mouse 6641*), the myocarditis had led to the formation of a large recent thrombus in the left ventricle.

The *pulmonary lesions* were extensive. They were in the form of large, often confluent patches of consolidation, composed of solid masses of large mononuclears or epithelioid cells, with many fragmenting lymphocytes and poly-

morphonuclears. Acid-fast bacilli were numerous in the lesions. There were areas of early necrosis but no frank caseation.

The *liver*, which was grossly enlarged, contained numerous focal collections of cells which differed in appearance from the more typical tubercles produced by *Mycobacterium X* and other strains. There was an agglomeration of closely packed distorted nuclei, some of which could be identified as belonging to polymorphonuclears. Large pale epithelioid cells were not conspicuous. Bacilli were abundant in the lesions. No necrosis, caseation, or giant-cell formation were observed.

The *kidneys* were severely affected. Large tuberculous areas with myriads of bacilli were present in both cortex and medulla (figure 5). Again many polymorphonuclears took part in the reaction, although the bacilli were taken up exclusively by large mononuclear elements. An interesting finding was a group of tubules just beneath the surface which were greatly distended by masses of closely packed bacilli. The flattened epithelium was undamaged by their presence and there was no inflammatory reaction.

Two mice inoculated intracerebrally and killed after seven and eighteen days showed during life definite signs of encephalitis. There were found not only a severe meningitis, extending into the spinal cord, but large areas of necrosis deep in the mid-brain. In addition, one of the mice had necrosis of the Purkinje cells associated with the presence of acid-fast granules, as has been described in several mice infected with *Mycobacterium X*. After eighteen days, dissemination had occurred and there were early tubercles in the liver and spleen.

Comparing these lesions with those produced by *Mycobacterium X*, there is an obvious difference in extent and severity. The severe involvement of the lungs, myocardium, and kidneys caused by the Vallée strain was never found with the *Mycobacterium X*.

Lesions produced by the vole bacillus: Sixteen mice, infected by various routes and killed at intervals from seven to thirty-five days, were studied. The lesions in their distribution and histopathology closely resembled those caused by *Mycobacterium X* and need not be described in detail. Only two differential points were observed. The bacilli themselves were more slender and often terminated in a hooked end; they showed little tendency to assume granular forms in the tissues.

Another feature which was not observed with other strains was the formation of a subcutaneous nodule in the abdominal wall at the site of injection. This was composed of a central mass of large mononuclears loaded with acid-fast bacilli and a wide capsule of fibroblasts. Individual bacilli occurred within the fibroblasts. Similar focal lesions have been described by Pagel after subcutaneous injection of virulent bovine strains into mice (5).

Lesions produced by the Sheard strain of avian tubercle bacilli: Nine mice, injected by various routes, were sacrificed at intervals of seven to sixty-three days after inoculation.

After intravenous inoculation, progressive and severe lesions were found in spleen and liver; the lungs were less severely affected. These consisted of massive accumulations of pale epithelioid cells which, in the liver, crowded apart the

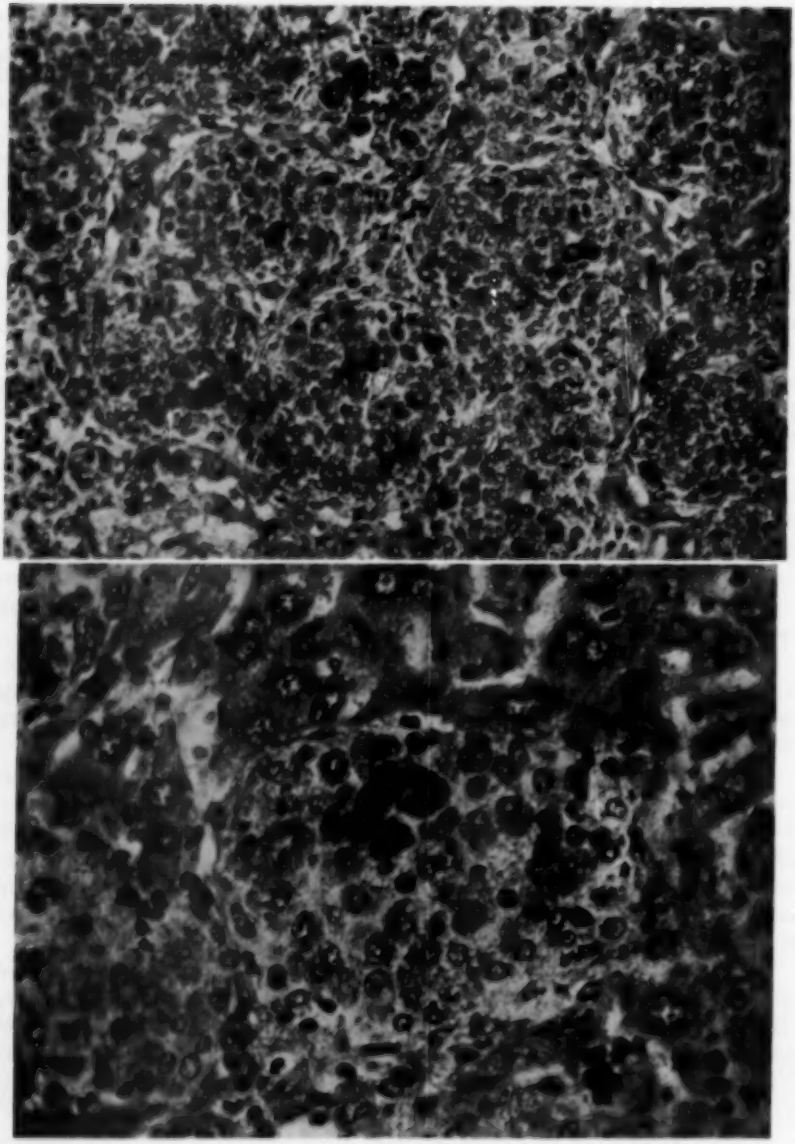

FIG. 6. *Mouse 6631.* (Top). Inoculated intravenously with Sheard avian strain. Killed after 63 days. Liver largely replaced by macrophages filled with acid-fast bacilli. Ziehl-Neelsen (×350)

FIG. 7. *Mouse 6631.* (Bottom). Liver macrophages filled with acid-fast bacilli but showing no degenerative changes. Ziehl-Neelsen (×700)

parenchymal cells and, in the spleen, largely replaced the normal elements of the pulp. There were no discrete tubercles, no necrosis, no giant-cell formation, and no conspicuous peripheral lymphocytic reaction. The proliferating cells contained vast numbers of acid-fast bacilli which often formed dense intracellular colonies. Remarkably enough, their growth appeared to have little deleterious effect upon the host cells, judging from the normal appearance of their nuclei. Granular forms of the bacilli were rarely observed.

Particularly interesting lesions were found in *Mouse 6631*, killed sixty-three days after inoculation. The liver and spleen were grosssly much enlarged and pale. Histologically, the liver tissue was found to be largely replaced by closely agglomerated, roughly circular tubercles, composed of large pale cells filled with massive numbers of well-stained acid-fast bacilli (figures 6 and 7). The same type of tubercles had largely replaced the splenic pulp and the intra-abdominal lymph nodes. The lungs showed a diffuse tuberculous arteritis; often there were heaped up masses of large mononuclears projecting into the lumen; acid-fast granules and short rods could be demonstrated within the cells. About each vessel was a broad cuff of lymphocytes. Only an occasional tubercle was seen in the parenchyma.

In the fatty capsule of the kidney was a series of discrete tubercles filled with acid-fast bacilli. The microorganisms could be seen to lie within individual fat cells (figure 8).

Following intracerebral inoculation, a fairly severe and progressive meningitis and ventriculitis ensued; the most distinctive feature was the massive intracellular multiplication of the bacilli without apparent damage to the phagocytic cells. In animals allowed to survive for three or four weeks, visceral dissemination had occurred but, in contrast to the diffuse reaction following intravenous inoculation, the lesions were small and discrete, in the form of isolated tubercles.

In summary, the Sheard avian strain causes no obvious signs of illness in the living mice, but establishes itself in the tissues and multiplies in an unlimited way within the phagocytic cells, apparently without cyto-pathogenetic effect. This unrestrained symbiotic growth of the bacilli readily differentiates the lesions from those caused by *Mycobacterium X*.

Lesions caused by an avirulent strain of avian tubercle bacilli (A 1): Seven mice were inoculated, 4 intravenously, 3 intracerebrally. The lesions were discrete in liver and spleen, and the number of bacilli and granular forms were relatively small in comparison with those in the lesions produced by the Sheard strain. After intracerebral inoculation, no visceral dissemination occurred, and at sixty-three days the bacilli had completely disappeared.

In general, the pathology was not strikingly different from that caused by *Mycobacterium X*, and it was not possible to identify any differential features.

Lesions caused by the murine leprosy bacillus: An opportunity was afforded to study livers and spleens of 20 mice which had been inoculated intraperitoneally with spleen suspensions containing the Hawaiian strain of murine leprosy bacilli and to compare the lesions thus induced after thirty and forty-three days with those following infection with *Mycobacterium X*. There were certain differences.

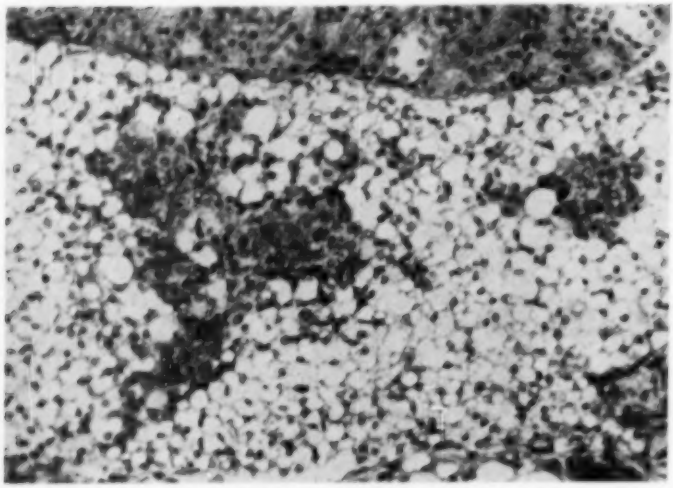

FIG. 8. *Mouse 6631.* Peri-renal adipose tissue with masses of macrophages and fat cells containing numerous acid-fast bacilli. Ziehl-Neelsen (×350)

In the liver, definite tubercle formation was exceptional. Most of the lepra bacilli were contained within Kuppfer cells and had excited no reaction. The focal cellular accumulations were poor in large epithelioid cells and included a fair proportion of polymorphonuclears. In a few of the focal lesions, large cells stuffed with bacilli were conspicuous.

In the spleens, some of which were obviously enlarged—others not—clumps of bacilli were found in scattered individual reticulo-endothelial cells. There were no tubercles or focal collections of epithelioid cells, such as were frequently found in the *Mycobacterium X* lesions.

The bacilli themselves were more slender than the *Mycobacterium X*, and coarse granular forms were not seen.

Pathogenicity of Mycobacterium X for Other Laboratory Animals

Guinea-pigs: Mycobacterium X has very slight pathogenicity for guinea pigs. Four animals were inoculated; none showed signs of illness. A brief summary of the pathologic changes noted follows:

G. P. 6296. Received 0.5 ml. of a ten-day culture subcutaneously. It was killed after thirty-five days. There were no gross lesions. The inguinal lymph node draining the site of injection was slightly enlarged and contained a few small nodules of epithelioid cells. No bacilli or granules were found in these lesions, but some of the cells contained large clumps of acid-fast material, possibly derived from the breakdown of the bacilli. There were no tuberculous lesions of the viscera. Cultures from the lung on Dubos medium remained sterile.

G. P. 6634. Injected intracardially with 1.0 ml. of a 10-day culture on Tween®-albumin medium. Sacrificed after five weeks. Grossly, the lung showed extensive patches of grayish

consolidation. Microscopically, extensive partial consolidation was found with proliferation and desquamation of alveolar cells, marked septal thickening, and, in some areas, polymorphonuclears in alveoli and small bronchi. No bacteria could be demonstrated in Gram- or Ziehl-Neelsen-stained preparations. The cause of the pneumonic lesions was not disclosed, but there was no reason to regard them as tuberculous.

The liver contained a few tubercles, composed of a few large epithelioid cells, sometimes multinucleate, and a peripheral ring of lymphocytes. A few small deeply stained acid-fast granules were seen and larger clumps of less intensely stained acid-fast material. There were no bacillary forms. The spleen showed patchy enlargement of the reticular cells but no definite tubercles. There were clumps of acid-fast pigment but no bacilli. Cultures were not taken.

G. P. 6436. Injected intracardially with the same dose as *G. P. 6634* but allowed to live for seven and one-half weeks. Again, there were no gross lesions. The liver contained many tubercles of the type described, but no acid-fast structures could be seen. The spleen also contained scattered tubercles without bacilli or granules. Cultures from liver, spleen, and lungs were negative.

G. P. 6498. Injected intraperitoneally with 1.0 ml. of a ten-day culture; killed after ten weeks. There were no gross lesions. Microscopically, the peritoneal fat showed a granulomatous reaction but no acid-fast bacteria. A single tubercle was found in the adrenal cortex, and scattered ones were noted in the lungs. The liver, spleen, and intraperitoneal lymph nodes were free. No cultures were taken.

In summary, guinea pigs react to *Mycobacterium X* by the formation of small tuberculoid nodules. The bacilli disappear rapidly from these lesions, and cannot be found in sections or recovered in cultures.

Rabbit 6463. Received 1.0 ml. of a ten-day culture of *Mycobacterium X* intravenously and was killed after two months. Unfortunately, this animal proved to have coccidial infection of liver and bile passages and another complication in the form of large areas of encapsulated necrosis, in which could be seen transections of an unidentified verminous parasite.

There were, however, lesions which presumptively could be associated with the mycobacterial infection. There were a number of small and rather ill-defined tubercles in liver and spleen in which no acid-fast bacilli or granules could be demonstrated but which were identical with those produced in guinea pigs.

An interesting observation which deserves recording, even if its interpretation is obscure, is the presence in the cytoplasm of many liver cells, of scattered acid-fast granules. They were not very numerous and there might be only one or two in a cell. They were not found in the Kupffer cells. Although they could not be distinguished morphologically from minute granular forms of *Mycobacterium X*, one hesitates to identify them as such.

Based on experience with this single animal, it may be inferred that *Mycobacterium X* has negligible pathogenicity for this species.

Chickens. Two full-grown chickens were inoculated. One received 1 ml. of suspension of a ten-day Tween®-albumin culture intravenously and was killed forty-two days later; the other was inoculated with 1 ml. intraperitoneally and was sacrificed after three months. Neither showed signs of illness and there were no gross lesions. Microscopically, there were scattered small tuberculoid nodules in liver and spleen, but these contained no acid-fast bacilli or granules. There were, however, clumps of pale, fuchsinophilic material, possibly derived from disintegrated bacilli.

From the experience with these two animals, it appeared that *Mycobacterium X* was nonpathogenic for chickens.

DISCUSSION

The pleomorphic acid-fast bacilli, isolated from mouse brain and provisionally called *Mycobacterium X*, has proved to have limited pathogenicity for white mice on experimental infection. None of the inoculated animals has shown obvious evidence of illness, and the mortality over a period of several months has been zero.

On the other hand, the bacilli do provoke a tissue reaction in the liver, lymph nodes, and adipose tissue, rarely and to a very slight degree in lungs and spleen. After intracerebral inoculation, a subacute or chronic leptomeningitis is elicited in which acid-fast organisms persist for at least two months.

The histopathology is a simple one. The bacilli are taken up by large macrophages which form small agglomerations in the organs and are surrounded by a zone of small lymphocytes. There appears to be little toxic effect upon the cells; necrosis or caseation never occurs, and there is no formation of giant cells. In the meningeal and adipose tissue, granulomatous reaction is more diffuse and isolated tubercles are of rare occurrence.

Under conditions which have not yet been clearly analyzed, the acid-fast organisms in the lesions assume granular forms and bacilli may be completely absent. It has been shown in an accompanying paper (Paper I) that, in tissue cultures of monocytes, the granules may evolve into bacillary forms. Whether a similar evolution may take place in the tissue lesions is difficult to prove. Transitional forms between granules and rods are often seen, but the direction of the change cannot be inferred from what is seen in the fixed tissue. It seems probable, however, from the work of Kahn and Nonidez (5), Wycoff (6), and others on living organisms that granules may regenerate into bacilli.

Brieger and associates (7) were able to obtain, from rabbit lungs and spleens seven to twenty-one days after intravenous infection with bovine bacilli, suspensions composed exclusively of granules. This was accomplished by using the technique of Fulton and Begg for purification of rickettsia and Niggs virus-containing tissue. When such granular suspensions were injected subcutaneously into guinea pigs, a local abcess in the regional lymph node resulted, later followed by generalized infection. Bacillary forms were abundant in the lesions. From these observations, it is concluded that infection may be produced by larger granular forms.

Lack and Tanner (8) have also recently studied the pleomorphic forms of human tubercle bacilli. In smears from tuberculous bone lesions they frequently observed acid-fast coccal forms, in the absence of bacilli. They attempted to bring about this change by varying the environmental conditions. As a result of study with the phase microscope and the use of malachite green to distinguish living bacteria from the dead forms stained by saffranin, they concluded that there are three types of granules. The three types are: nuclear remains of auto-

lyzed bacilli; detachment of growing tips of bacilli in smearing (artifact); and granular forms formed by division of diphtheroid forms under adverse conditions. These granules stain with malachite green and are assumed to be capable of reproduction and of evolving into bacillary forms.

Whatever the ultimate significance of these granular acid-fast bodies, it would seem that their occurrence in the lesions of the infected mice of the present study are characteristic, if not pathognomonic, of this particular strain. Lesions associated solely with granular bodies were not observed with the other strains which were studied.

Guinea pigs, rabbits, and chickens, so far as the limited experiments indicate, are almost completely resistant to this mycobacterial strain. Although small tuberculoid lesions may be found in the liver after intravenous injection, stainable bacilli rapidly disappear from the lesions and the animals do not become ill.

Only a few references have been found in the literature concerning the spontaneous occurrence of mycobacterial infection in mice. One of the earliest references is to a paper by De Jong (10). From three white mice inoculated with material from nontuberculous pigs, acid-fast bacilli were recovered from the lungs. Because of their moist growth ("cultures humides") and their limited pathogenicity for guinea pigs, they were regarded as probably avian in type. There were no histologic studies.

In 1904, Rabinowitch (11) examined 88 gray mice, 16 of which she found to be infected with avian-type tubercle bacilli. Liver and spleen were chiefly affected. The author believed that the mice might have been infected by the feces of the fowl. Simmons (12), in 1927, isolated from the intestinal contents of a house mouse acid-fast bacilli which proved to be nonpathogenic for mice, guinea pigs, or rabbits. For this strain, which grew rapidly on Petroff's medium, he suggested the name *Mycobacterium tuberculosis* var. *muris n. sp.*

Hemmert-Halswick (13), in 1934, described a lepra-like disease occurring as a spontaneous infection of white mice produced by an acid-fast bacillus, which the writer believes is an avian tubercle bacillus. There were cutaneous and visceral lesions. Growth was obtained on ox-blood agar plates and glycerin potato, Petroff's and Löwenstein's media, and on Besredka's glycerin bouillon. The typical disease in mice could be reproduced with pure cultures. There was limited pathogenicity for guinea pigs.

In view of the resemblance of *Mycobacterium X* to strains of avian bacilli of low virulence, it is interesting to note that three of the four writers who are cited regarded the strains isolated by them from mice as avian in type, although this conclusion was based on criteria which today might not be regarded as convincing.

SUMMARY

A mycobacterium isolated from mouse-brain suspensions was found in experimental inoculations to have low pathogenicity for mice and virtually none for guinea pigs, rabbits, or chickens. The lesions were in the form of microscopic tubercles found predominantly in liver and intra-abdominal lymph nodes, rarely

and sparsely in spleen and lungs. The most interesting observation is that some of the lesions were associated with the exclusive presence of acid-fast granules.

Resemblances and differences of the lesions when compared with those produced by certain other strains of mycobacteria are discussed.

SUMARIO

Observaciones sobre una Micobacteria Pleomorfa Aislada de Cerebros de Ratones: II. Histopatología y Comparación de las Lesiones con las Producidas por Ciertas Otras Cepas de Micobacterias

Una micobacteria aislada de suspensiones de cerebros de ratones se mostró, en inoculaciones experimentales, dotada de poca patogenicidad para los ratones y de virtualmente ninguna para cobayos, conejos o pollos. Las lesiones tomaron la forma de tubérculos microscópicos que se encontraron predominantemente en el hígado y los ganglios linfáticos intra-abdominales y rara y escasamente en el bazo y los pulmones. La observación más interesante fué que algunas de las lesiones estaban asociadas con la presencia exclusiva de gránulos ácidorresistentes.

Discútense las semejanzas y diferencias de las lesiones al ser comparadas con las producidas por ciertas otras cepas micobacterianas.

RESUME

Observations sur une mycobactérie pléomorphique isolée du cerveau de souris. II Anatomopathologie et comparaison des lésions avec celles produites par d'autres souches de mycobactéries.

Une mycobacterie extraite de suspensions de cerveau de souris a démontré une pathogénicité réduite pour les cobayes, lapins et poulets au cours d'inoculations expérimentales. Les lésions se présentaient sous la forme de tubercules microscopiques qui étaient, le plus souvent localisés au niveau du foie et des ganglions lymphatiques intra-abdominaux, ils étaient rares et dispersés dans la rate et les poumons. L'observation la plus intéressante est que certaines lésions étaient exclusivement associées à la présence de granules acido-résistants.

Les ressemblances et les différences entre les lésions par comparaison avec celles produites par d'autres souches de mycobactéries sont discutées.

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EFFECTS OF SUNLAMP IRRADIATION ON THE VIABILITY, VIRULENCE, AND BIOCHEMICAL ACTIVITY OF MYCOBACTERIUM TUBERCULOSIS[1, 2]

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INTRODUCTION

On the basis of a preliminary finding that exposure of a glass-sealed suspension of virulent tubercle bacilli (strain H37Rv) to sunlight for one hour had a pronounced effect on its viability, but an even greater effect on its pathogenicity, it was decided to investigate the effects of sunlight on the virulence of *Mycobacterium tuberculosis*. Furthermore, the use of sunlight as an experimental tool was indicated by occasional references in the literature to the high susceptibility of the tubercle bacillus to the harmful effects of sunlight. More recently, the attenuation of BCG vaccines has been attributed to exposure to sunlight (1).

The present paper describes some effects of sunlamp irradiation on the viability, virulence, and biochemical activity of *M. tuberculosis*, with particular reference to a comparison of the effects on strains and cultures which differ in their degree of virulence.

MATERIAL AND METHODS

Strains of M. tuberculosis: The following three strains representative of different degrees of virulence were used: H37Rv (virulent),[3] BCG "Phipps" (attenuated)[4], and H37Ra (avirulent).[3]

Methods of culture: Tween®-albumin liquid medium (2) was used for submerged growth of the bacteria. Unless otherwise stated, twelve-day-old cultures were used. When amithiozone (para-formylacetanilide thiosemicarbazone) was included in the Tween®-albumin liquid medium, it was in a concentration of 0.5 γ per ml. Further details of these and other methods referred to in this paper may be found in a previous paper (3).

Preparation of bacterial suspensions: After growth in submerged culture, the bacterial cells were centrifuged, washed twice in a salt solution containing Tween® 80 (the Tween®-albumin medium without any of the organic constituents), and finally suspended in this same solution to standard optical density using a Coleman Sr. spectrophotometer. For a quantitative estimate of cell amounts, either dry weight determinations of water-washed cells or plate counts (4) of viable numbers were used. The values for these determinations are given in the results of each experiment.

Conditions of irradiation: Ten-milliliter samples of cell suspension were added to glass-covered storage dishes (diameter 100 mm., height 80 mm.) making a liquid layer 6 mm. in

[1] From the Division of Tuberculosis, The Public Health Research Institute of The City of New York, Inc., New York, New York.

[2] This study was carried out under the terms of a cooperative agreement between this Institute and the Field Research Branch, Division of Chronic Diseases and Tuberculosis, Public Health Service.

[3] Obtained through the courtesy of Mr. W. Steenken, Jr., the Trudeau Laboratories, Trudeau, New York.

[4] Obtained through the courtesy of Dr. R. J. Dubos, The Rockefeller Institute for Medical Research, New York, N. Y.

depth. The dish was gently agitated by means of an adjustable shaking machine at the rate of 50 double strokes per minute. The temperature of the cell suspension was controlled at 25°C. by means of a fan placed at a distance of one and one-half feet from the dish. The entire operation was carried out in an air-cooled room. The source of light was a G.E. 100 watt S-4 sunlamp, which emits radiation comparable to the ultraviolet radiation of sunlight (approximately 2,800 to 3,970 Angstrom units), as well as visible radiation (3,970 to 7,594Å). When a glass cover was used over the dish containing the cell suspension, as in most of the experiments, wave lengths shorter than 3,100Å were filtered out. This approximately corresponds to the limiting wave length of sunlight that penetrates to the earth in the winter or in smoky cities (5). The S-4 sunlamp was held in an aluminum painted dome-shaped, adjustable reflector attached to a floor lamp containing the G.E. transformer necessary for operation of the S-4 sunlamp. The sunlamp was held at an angle of 45 degrees above the dish containing cell suspension and at a distance of 1 cm. above the cover of the dish. The sunlamp was rinsed in 95 per cent alcohol before use and wiped clean with a chamois cloth. The dishes used to hold the cell suspension were also kept meticulously free of dust and markings. The sunlamp was allowed to burn for ten minutes to allow for full output prior to actual irradiation of cell suspensions. Irradiation was carried out at specific time intervals given in the results of the individual experiments. Following irradiation and in subsequent handling, the cell suspensions were shielded from room light.

In experiments where ultraviolet irradiation was used, the cell suspensions were exposed in the open storage dishes held on a revolving turntable at a distance of 16 cm. below three germicidal G.E. 15 watt lamps (chiefly 2,537Å), all enclosed in a chamber.

Viability: Three indices of viability were used: (*1*) plate counts on oleic-acid–albumin agar (4); (*2*) hydrogen transfer capacity tested by reduction of 2,3,5 triphenyltetrazolium chloride (6); (*3*) respiration as measured by the rate of oxygen consumption in a Warburg apparatus, where the bacterial cells were suspended in the regular Tween® medium without albumin, and with 0.5 per cent Tween®.

Virulence: Male albino mice of the CF1 strain[5] were injected intravenously with 0.1 ml. of bacterial suspensions. The severity of infection as measured by survival time was used as an index of virulence.

OBSERVATIONS

Relationship Between Virulence of Tubercle Bacilli and Their Susceptibility to Injurious Effect of Sunlamp Irradiation

Comparative effect of irradiation on H37Rv, H37Ra, and BCG strains of M. tuberculosis as measured by plate counts of viable numbers: The susceptibility of tubercle bacilli to the injurious effect of radiation was investigated by subjecting three strains, H37Rv, H37Ra, and BCG, to three different ranges of light waves for different periods of time. Three strains of various degrees of virulence were used in the hope that a difference in their reaction to light might be revealed which could be correlated with their virulence.

Washed cell suspensions of the three strains were irradiated as follows:
Sunlamp, glass-filtered (>3,100Å) at 4, 8, 12, 16, 24, and 32 minutes
Sunlamp, unfiltered (>2,800Å) at 2, 4, 8, 12, and 16 minutes
Germicidal lamp, unfiltered (2,537Å) at 30 seconds and 1, 2, 4, and 8 minutes.

All samples were plated out on oleic-acid-albumin agar, and counts of the colonies were made after three weeks' incubation at 37.5°C. Nonirradiated controls of the three strains were plated out to give a quantitative estimate of original cell numbers in the suspensions to be irradiated.

[5] From Carworth Farms, New City, New York.

The results are summarized in figures 1 and 2. The survival curves in figure 1 are based on the logarithms of the surviving numbers as estimated by an average of triplicate plate counts. Several conclusions may be drawn from the results presented in figure 1.

A very marked lethal effect of irradiation was evident for all three strains of tubercle bacilli. Although reduction in viable numbers was maximal at the known germicidal wave length of 2,537Å, the near-visible ultraviolet and visible

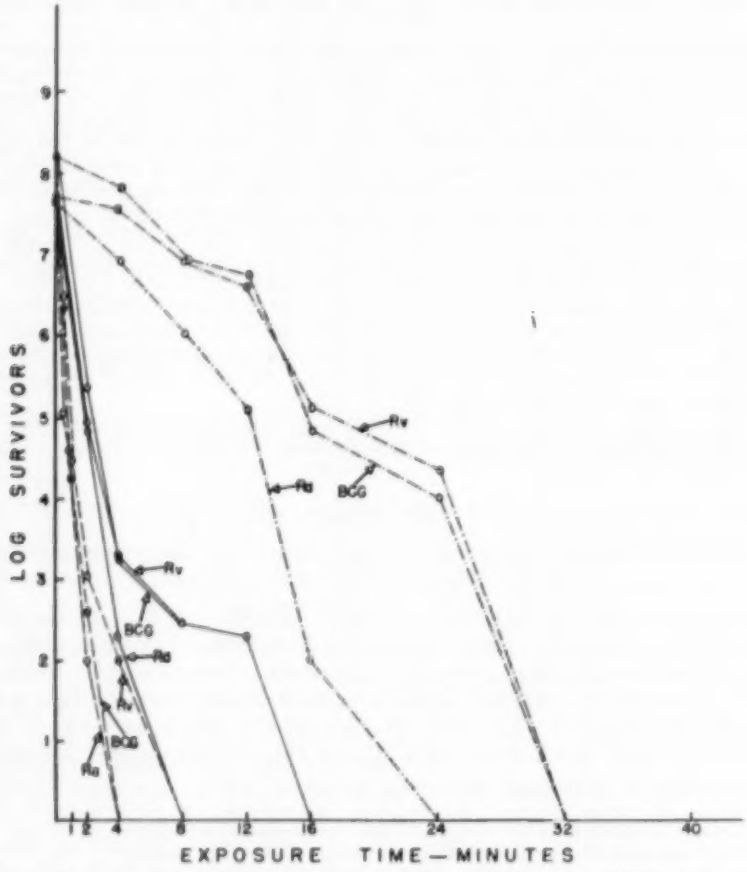

FIG. 1. Comparison of dose-survival curves of H37Rv, H37Ra, and BCG-Phipps irradiated at three different ranges of wave lengths.

0- · - · -0 > 3,100Å
0————0 > 2,800Å
0 - - - - 0 2,537Å

Original viable numbers per ml. of cell suspension before irradiation: H37Rv: 5.1×10^7; H37Ra: 4.4×10^7; BCG: 1.6×10^8. Values represent averages of triplicate plate counts.

wave lengths of light (>3,100Å) showed considerable activity toward all three strains.

A definite differential rate of killing was in evidence for the three strains, which varied in degree of virulence. The avirulent strain, H37Ra, was found to be most susceptible to the germicidal effect of light; the attenuated strain, BCG, was intermediate; and the virulent strain, H37Rv, showed greatest resistance. This scale of susceptibility was evident for all three ranges of wave lengths. Consistent with this effect was the fact that the log survival curve for H37Ra approached a straight line for irradiation at the middle ultraviolet wave length

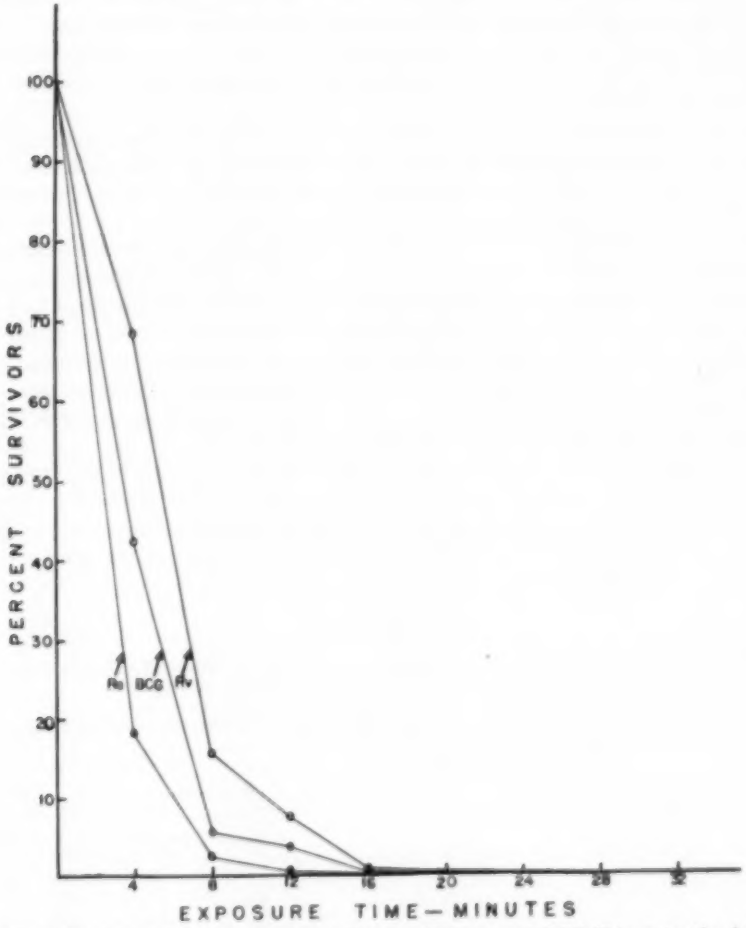

FIG. 2. Comparative susceptibility of H37Rv, H37Ra, and BCG-Phipps to the lethal effect of sunlamp irradiation (>3,100Å). The same values of viable numbers shown in figure 1 were used, but expressed on a percentage basis.

of 2,800Å, whereas the curve for irradiation of H37Rv at the far ultraviolet wave length of 2,537Å still showed some threshold characteristics. The differential effect of irradiation on different degrees of virulence was most pronounced at the least germicidal wave lengths, >3,100Å. The latter results are more clearly illustrated in figure 2, where the discrepancy in original numbers of the three strains has been avoided by plotting the percentage of surviving bacilli against exposure time.

Comparative effect of irradiation on H37Rv, H37Ra, and BCG strains of M. tuberculosis as measured by hydrogen transfer capacity: The differential scale in the susceptibility of virulent, attenuated, and avirulent strains of *M. tuberculosis* to irradiation was corroborated by measuring the effect on viability using 2,3,5 triphenyltetrazolium chloride. This dye, which measures the hydrogen transfer capacity of a culture, has been shown to give good estimates of viability for tubercle bacilli (6).

In this experiment and in all others to be reported in this paper, irradiation was carried out by the sunlamp with a glass cover over the dish containing the cell suspension, so that the radiation was limited to 3,100Å and over. The results are presented in table 1.

From the nonirradiated controls, it is seen that the hydrogen transfer capacity was greatest for H37Ra (10 per cent T), was less for BCG (20 per cent T), and was least for H37Rv (28 per cent T). This is consistent with previous findings regarding the rate of reduction of methylene blue by different strains of tubercle bacilli (7). Taking this inherent difference of the three strains into account, it is seen from the results in table 1 that the rate of decrease in hydrogen transfer capacity, as a result of increased time of irradiation, was greatest for H37Ra, was intermediate for BCG, and was least for H37Rv.

In terms of viability, these results indicate that the avirulent strain, H37Ra, was subject to the greatest rate of reduction in viable numbers and, hence, was most susceptible to the injurious effect of sunlamp irradiation; the attenuated strain, BCG, was intermediate in its susceptibility; and the virulent strain, H37Rv, was most resistant to irradiation. These results are consistent with

TABLE 1

COMPARATIVE EFFECT OF S-4 SUNLAMP IRRADIATION ON THE HYDROGEN TRANSFER CAPACITY OF CELL SUSPENSIONS OF H37Rv, H37Ra, AND BCG

Irradiation Time (Minutes)	Per Cent Transmittance of Reduced 2,3,5 Triphenyltetrazolium Chloride		
	H37Ra*	BCG*	H37Rv*
0	10	20	28
4	40	45	37
8	61	58	42
12	62	74	60
16	91	80	69
24	88	84	87

* Approximately equal amounts of cells were used, estimated as dry weight: Ra (1.6 mg.), BCG (1.5 mg.), Rv (1.6 mg.) per tube.

those shown in figures 1 and 2, in which plate counts were used as a measure of viability. Hence, the results confirm the differential scale in susceptibility to the effect of irradiation as correlated with degree of virulence of the tubercle bacillus.

Comparative effect of irradiation on viable numbers of cultures of varied virulence of the H37Rv strain of M. tuberculosis: The correlation between susceptibility to light and virulence was further explored by testing cultures of H37Rv which differed in their physiologic state. It has been shown (8, 9) that young cultures of H37Rv are more virulent than older ones. It has also been demonstrated (3) that, when H37Rv is grown in the presence of 0.5 γ of amithiozone per ml., the virulence as well as the production of cord factor are considerably reduced, although the growth rate is unaffected. On the basis of these findings, H37Rv cultures of various ages and cultures grown in the presence of amithiozone were compared as to their susceptibility to irradiation. Again, it was found that susceptibility to the injurious effect of irradiation was correlated with degree of virulence. The results are summarized in table 2.

The five-day-old, most virulent of these cultures showed the greatest resistance to irradiation with the least reduction in viable numbers. The less virulent cultures, aged twelve and twenty-one days, were considerably more susceptible to irradiation, as evidenced by a greater reduction in viable numbers at the three exposure times. The H37Rv culture grown in the presence of amithiozone, the virulence of which was greatly reduced (3), showed the greatest susceptibility to light. In fact, the degree of susceptibility of this culture was of the order of magnitude found for the avirulent strain, H37Ra. Thus, the correlation of susceptibility to the lethal effect of irradiation and degree of virulence held not only for strains differing in their degree of virulence but also for cultures of the same strain which differed in their state of virulence.

TABLE 2

COMPARATIVE EFFECT OF SUNLAMP IRRADIATION ON VIABLE NUMBERS OF CULTURES OF H37Rv WHICH VARY IN THEIR DEGREE OF VIRULENCE

Irradiation Time (Minutes)	Per Cent Reduction in Viable Numbers*			
	Age of Culture (Days)†			Grown in Presence of Amithiozone‡
	5	12	21	
6	36.4	63.8	56.90	94.40
12	51.5	85.1	98.40	99.70
24	72.7	99.3	99.96	99.99

* Values represent averages of triplicate plate counts.

† Nonirradiated controls gave the following values of original viable numbers per ml. of washed and adjusted cell suspension:

5-day-old cultures: 33×10^6
12-day-old cultures: 47×10^6
21-day-old cultures: 58×10^6

‡ Five-day-old cultures containing 0.5 γ of amithiozone per ml. were used. The nonirradiated control of these cells contained 43×10^6 viable numbers per ml. of adjusted suspension.

Effect of Sunlamp Irradiation of H37Rv on Its Virulence for Mice

The initial observation that exposure of a suspension of H37Rv to sunlight for one hour resulted in a reduction in its virulence for mice, disproportionate to the reduction in viable numbers, was further investigated.

Groups of 15 mice each were injected intravenously with equal suspensions of six-day-old cultures of H37Rv which had been previously exposed to the sunlamp at one-, two-, four-, eight-, and sixteen-minute time intervals. Control groups of mice were injected with original nonirradiated cell suspension, as well as original cell suspension diluted by one-third and two-thirds, so that the viable numbers in these dilutions might be comparable to the reduced viable numbers resulting from irradiation. As it worked out, the one-third dilution of the original cell suspension was by chance exactly equal to the number of viable cells remaining in the suspension irradiated for sixteen minutes.

To exclude the possibility that the dead bacilli present in the irradiated suspension along with the viable bacilli might be the cause of observed lowered virulence by virtue of possible immunizing activity, a further control experiment was done at a later date. A group of mice was injected with a cell suspension containing a mixture of living and dead bacilli. However, in this case, the living bacilli were not exposed to the sunlamp, and the dead bacilli

TABLE 3

EFFECT OF SUNLAMP IRRADIATION OF H37RV ON ITS VIRULENCE FOR MICE

Irradiation Time (Minutes)	Viable Numbers*	Survival Time (Days) After Infection (15 Mice per Group)	Median Survival Time (Days)
0	14×10^5	17,18,18,18,19,19,20,21,21,21, 22,22,8,8,8†	21
1	12×10^5	15,17,17,17,18,19,19,20,20,20, 21,23,28,8,8	20
2	11×10^5	16,18,18,18,18,19,19,20,20,21, 21,22,32,8,8	20
4	7.4×10^5	17,20,21,21,21,22,22,26,45,47, 55,58,69,71,8	26
8	6.2×10^5	20,20,21,22,24,28,42,44,47,49, 54,8,8,8,8	44
16	4.7×10^5	30,33,61,61,62,63,75,8,8,8,8,8, 8,8,8	>78
Controls			
Two-thirds dilution (nonirradiated)	9.3×10^5	18,18,18,19,19,19,19,20,20,20, 20,21,22,58,8	20
One-third dilution (nonirradiated)	4.7×10^5	20,20,20,21,21,22,22,22,22,22, 23,48,74,8,8	22
Mixture of nonirradiated viable and irradiated dead cells (1:2)	11.6×10^5	16,16,17,17,17,17,18,18,19,19, 19,19,19,19,20,20,21,22,22‡	19
Same number, nonirradiated viable cells alone	11.6×10^5	17,18,18,18,18,19,19,20,20,20, 20,20,21,21,21,21,23,28,32‡	20

* Values represent averages of triplicate plate counts, except for the one-third and two-thirds dilutions where values are estimated.

† 8 signifies mouse was sacrificed 78 days after infection.

‡ Twenty mice per group.

were killed separately by irradiation. The two suspensions were then mixed, the proportion of living to dead bacilli corresponding to that found in the sixteen-minute irradiated suspension, namely, 1:2.

The results of the entire experiment are summarized in table 3. Sunlamp irradiation of H37Rv for sixteen minutes resulted in a marked reduction in the virulence of the surviving bacilli. The actual reduction in viable numbers (two-thirds) as a result of irradiation would not in itself be a factor in the observed reduction in virulence, for the nonirradiated control, diluted (one-third) to corresponding viable numbers, showed no significant reduction in virulence. That the observed effect was not due to possible immunizing activity on the part of the dead cells included in the injection of the sixteen-minute irradiated suspension was shown by the results of the control mixture of nonirradiated viable and irradiated dead cells, which was no less virulent than an equal number of viable cells alone.

It may thus be concluded that irradiation of tubercle bacilli reduces their pathogenicity without affecting their viability as measured by plate counts. Furthermore, the effect appears to be proportional to the dose, since irradiation for four minutes showed a little reduction in virulence, eight minutes showed considerably more, and sixteen minutes of exposure resulted in the greatest reduction.

Comparative Effect of Sunlamp Irradiation of H37Rv on Its Reduction in Rate of Respiration and Viable Numbers

In an attempt to obtain some further clue to the nature of the effect of sunlamp irradiation on H37Rv, the rate of respiration of irradiated suspensions was measured in the Warburg apparatus. It was found that the reduction in respiration rate with increased exposure time was surprisingly low in terms of what would be expected from the standard killing curves of irradiated H37Rv. In table 4 are compared the values of per cent reduction in viable numbers, representing typical results repeatedly obtained from irradiation of a twelve-day-old

TABLE 4

COMPARATIVE EFFECT OF SUNLAMP IRRADIATION OF H37RV ON ITS REDUCTION IN RATE OF RESPIRATION AND VIABLE NUMBERS

Irradiation Time (Minutes)	Per Cent Reduction in Viable Numbers*	Per Cent Reduction in Rate of Respiration†
4	31.4	2.0
8	84.3	20.3
12	92.2	14.7
16	99.7	66.8
20	99.9	81.0

* Values represent averages of triplicate counts. Original numbers per ml. of nonirradiated control: 5.1×10^7. Plate counts were not made on the same cell suspensions used to measure respiration.

† Values represent averages of triplicate determinations of cu. mm. oxygen uptake per hour per mg. dry weight.

TABLE 5

PROTECTIVE EFFECT OF BOVINE ALBUMIN ON IRRADIATED SUSPENSIONS OF BCG

Irradiation Time (Minutes)	Per Cent Reduction in Viable Numbers*	
	Without Albumin	In Presence of 10 Per Cent Albumin
4	67.2	5.8
8	99.8	74.5
16	99.9	81.0
24	100.0	99.6

* Values represent averages of triplicate plate counts. Original numbers per ml. of nonirradiated controls:

without albumin: 24.3×10^6
in presence of 10 per cent albumin: 22.6×10^6

culture of H37Rv, with the values of per cent reduction in oxygen consumption. As seen from these results, a striking differential effect of irradiation was evident with respect to reduction in viable numbers as contrasted to reduction in oxygen uptake; the latter lagged far behind at each of the exposure times.

Protective Effect of Bovine Albumin on Irradiated Suspensions of BCG

A suspension of a twelve-day-old culture of BCG was prepared as described in *Materials and Methods*, except for an additional washing to assure removal of the albumin present in the culture medium. The suspension was divided into two portions. To one portion, bovine albumin (fraction V) was added to give a final concentration of 10 per cent. To the other portion, which served as control, only additional salt solution was added equal to the volume of albumin so that the two suspensions remained equal in cell density. Samples of each of the two suspensions were irradiated by sunlamp for different exposure times. The results are presented in table 5. At four minutes of exposure to radiation, the protective action of albumin was most pronounced. However, it continued to reduce the lethal effect of sunlamp irradiation even at increased exposure times.

DISCUSSION

In addition to the differential effect on strains already pointed out, examination of the dose-survival curves for irradiated suspensions of tubercle bacilli indicates that a definite difference exists in the nature of the effect of the three ranges of wave lengths. Irradiation at the longer near-visible ultraviolet and visible wave lengths of light (>3,100Å) was characterized by a threshold type of curve. When the shorter wave lengths of 2,800 to 3,100Å were included, the curves began to approach a straight line. This was particularly true with H37Ra, which further pointed to the greater susceptibility of this avirulent strain to the lethal effect of light. At the germicidal ultraviolet wave length of 2,537Å, irradiation resulted in straight-line relationships between logarithms of surviving numbers and exposure time, except for the more resistant virulent strain, H37Rv, the curve of which still showed some threshold characteristic.

These differences in the reaction of *M. tuberculosis* to different wave lengths of

the spectrum are not unique for this organism, but seem to be more a function of the inherent differences of the wave lengths used. Similar results have been obtained by Hollaender (10) for *Escherichia coli*. It is also interesting to note that, although the known germicidal wave length of 2,537Å was most effective, the long ultraviolet and visible radiation (3,100–7,594Å) used throughout the present study evidenced a considerable germicidal effect on all three strains of tubercle bacilli tested. This emphasizes the general susceptibility of *M. tuberculosis* to the effects of light.

The different effects produced by the three ranges of wave lengths served the additional function of confirming the differences in susceptibility of strain to the lethal effect of radiation. At all three ranges of the spectrum, the scale of virulence of the strain, as correlated with the scale of susceptibility, is apparent although expressed in different degrees and forms. It is most clearly expressed at the longer, less lethal wave lengths, where the intermediate reaction of BCG is more apparent, as shown in figure 2. This difference in light susceptibility was not only exhibited by a difference in the virulence of the strain but also by cultures of the same strain physiologically different in respect to their degree of virulence. The findings suggest, therefore, that the reaction to light is significantly and intimately linked to some key substance or mechanism involved in the infectious process of the tubercle bacillus. There are only a few biologically important compounds that show definite absorption bands in the near-visible and visible region of the spectrum (10). Riboflavin is one such compound and has been suggested (10) to explain the effects of visible light on *E. coli*. In view of the known production of riboflavin by tubercle bacilli (11, 12), this compound might be directly involved in the observed effects of sunlamp irradiation. Furthermore, there is some evidence (11) which points to a possible differential degree of production of riboflavin by strains of tubercle bacilli which vary in their virulence. The attenuated human strain produces the most riboflavin; the virulent human strain, the least; and BCG, an intermediate quantity. These results offer a possible explanation for the results reported in this paper on the differential effect of light expressed by a difference in virulence of strain and culture. In any event, irradiation has served as an excellent tool in confirming the close relationship which has been shown (3, 8) to exist between age of culture, formation of cord factor, and degree of virulence for animals.

The marked effect of sunlamp irradiation in reducing the virulence of strain H37Rv for mice appears to be clear and definite from the results obtained. The negative result obtained for the control of nonirradiated viable bacilli mixed with irradiated dead bacilli precludes the possibility that the observed reduction in virulence is due to the immunizing activity of the dead cells included in the injected irradiated suspensions. Furthermore, results presented in a following paper (13) show that irradiated cells of BCG have little or no immunizing capacity. Thus, it may be concluded that the observed reduction in virulence is due to an actual effect of radiation on the surviving cells of strain H37Rv.

The nature of this effect remains obscure. However, the effect is most probably not by way of a mutation or mutations. One or several attenuated or

avirulent mutants or variants would not be expected to show any effect when introduced into an animal with a mass of unchanged virulent bacilli. The majority or entire mass of surviving bacilli must have been altered in some way by irradiation in order to explain their loss in virulence when injected into mice. Furthermore, the idea of a mass effect is supported by the following factors:

Wave lengths were used (>3,100Å) which are not those known to produce mutation (in the shorter ultraviolet spectrum around 2,537Å and also X-rays). Hollaender (10) cites evidence showing that wave lengths 3,500 to 4,900Å are non-mutational in character. Whether this also applies to the range 3,100 to 3,500Å is a question, but appears likely. The reduction in virulence seems to increase with increased time of exposure, suggesting direct photochemical effect on the mass of bacilli. The mechanism of this effect of sunlamp rays may be directly related to the infectious process.

The fact that such irradiated cells, reduced in their virulence, continue to grow on agar medium points to the specificity of the effect. Conceivably, some light reactive substance (or enzyme producing such a substance) essential for the display of pathogenicity could be specifically inactivated photochemically resulting in a loss of virulence. The results already discussed for the differential susceptibility of strains which vary in their degree of virulence tend to enhance this possibility. The possible role of riboflavin has already been discussed and equally applies here. However, it is equally possible that the effect of light on virulence is only indirect and that the bacilli are weakened or damaged by irradiation to a degree insufficient to prevent their growth on an agar plate, but sufficient to swing the balance of the infectious process in favor of the host.

As far as evidences of change in subcultures of sunlamp irradiated suspensions of H37Rv are concerned, some were obtained but their significance is questionable. From agar platings of irradiated suspensions or from directly irradiated agar plates previously seeded with a suspension of H37Rv, there would sporadically appear, sometimes in considerable numbers, colonies which closely resembled those characteristic of the avirulent strain. However, upon transfer of these colonies to Tween®-albumin medium and subsequent plating, only colonies characteristic of H37Rv were found. Acid-fast stains of the liquid subcultures of the avirulent-like colonies also showed the normal staining and cording properties of H37Rv. As the occurrence of such avirulent-like colonies was so sporadic and as their subculture resulted in typical growth of H37Rv, their further study was discontinued. Direct subcultures of heavy inocula of irradiated suspensions of H37Rv into Tween®-albumin medium also resulted in changes, such as in the rates of methylene blue reduction and respiration, which occurred sporadically and which were not in evidence on further subculture.

The differential effect of irradiation of H37Rv on its reduction in viability and respiration is consistent with results recently reported by Kelner (14) for ultraviolet irradiation of *E. coli*. However, it may be significant that the effect obtained for *M. tuberculosis* was not by irradiation at the germicidal wave length of the ultraviolet spectrum 2,537Å, but rather by irradiation at the near-visible and visible region of the spectrum, which is the photoreactivating region. In

this connection, it might be added that several attempts made to photoreactivate suspensions of H37Rv in the manner reported for *E. coli* and other organisms (15) were unsuccessful. Both the S-4 sunlamp and a 500 watt tungsten G.E. projection lamp were used as light sources for photoreactivation after initial inactivation by ultraviolet irradiation. This lack of evidence for photoreactivation of *M. tuberculosis* is not unique for this organism. Both *B. subtilis* (15) and *B. cereus* (16) have been reported to show no evidence of photoreactivation.

Whereas the effect of irradiation on virulence represents an alteration of the surviving bacilli, the differential effect on viability and respiration may involve only the "dead" cells whose growth process has been arrested by irradiation, but whose respiratory mechanism has survived. This is the basis of the effect found by Kelner for *E. coli* (14). It is within the realm of possibility, however, that, with sunlamp irradiation of H37Rv, the remaining viable cells in the irradiated suspension are so affected that their respiration is increased. If this were true, it would relate this phenomenon and the observed alteration of virulence and possibly offer a basis for explanation of the latter.

The finding that albumin protects BCG from the lethal effect of sunlamp irradiation is consistent with the generally assumed protective effect of albumin. Doubtless, in many cases in which tubercle bacilli have been reported to remain viable for a longer period of time when suspended in albumin (4) or sputum (5), the bacilli are being protected from the effects of light by albuminous material.

SUMMARY

Sunlamp irradiation of the tubercle bacillus produced several striking effects in relation to the viability, virulence, and biochemical activity of this organism.

A close correlation was obtained between the degree of virulence of strains of *M. tuberculosis* (H37Rv, BCG, and H37Ra) and their susceptibility to the lethal effect of sunlamp radiation, as well as to two more germicidal regions of shorter ultraviolet wave lengths. The less virulent the strain, the greater was its susceptibility to irradiation.

This same correlation was also found to apply to cultures of the same strain (H37Rv) physiologically differing in their degree of virulence. More avirulent cultures, by virtue of older age or by growth in the presence of paraformylacetanilide thiosemicarbazone, were more susceptible to the lethal effect of light.

Irradiation produced a pronounced reduction in the virulence for mice of the H37Rv strain. The reduction in virulence appeared to be in proportion to the dose of irradiation and probably represents a direct photochemical alteration of the mass of bacilli injected into mice.

A given dose of irradiation produced a much greater reduction in viable numbers than in the rate of respiration. This result is considered in relation to photoinactivating and photoreactivating phenomena which have been described for *E. coli*. It is also considered in relation to the observed effect of light on reduction in virulence.

Albumin was shown to have a pronounced protective effect on suspensions of BCG exposed to a lethal dose of sunlamp radiation.

SUMARIO

Efectos de la Irradiación con la Lámpara Solar sobre la Viabilidad, Virulencia y Actividad Bioquímica del Mycobacterium Tuberculosis

La irradiación del bacilo tuberculoso con la lámpara solar produjo varios efectos notables en relación con la viabilidad, virulencia y actividad bioquímica de dicho microbio.

Obtúvose íntima correlación entre la intensidad de la virulencia de las cepas del *M. tuberculosis* (H37Rv, BCG y H37Ra) y su susceptibilidad al efecto letal de la radiación con la lámpara solar, así como de las dos regiones más germicidas de las ondas ultravioletas más cortas. Mientras menos virulenta la cepa, mayor era su susceptibilidad a la irradiación.

Se descubrió además que la misma correlación regía con cultivos de la misma cepa (H37-Rv) que discrepaban fisiológicamente en la intensidad de su virulencia. Los cultivos más avirulentos, por virtud de mayor edad o desarrollo en presencia de tiosemicarbazona paraformilacentanilídica, eran más susceptibles al efecto letal de la luz.

La irradiación produjo una reducción acentuada de la virulencia de la cepa H37Rv para los ratones. Esa reducción pareció ser proporcional a la dosis de irradiación y representa probablemente una alteración fotoquímica directa de la masa de bacilos inyectada en los ratones.

Una dosis dada de irradiación produjo una reducción mucho mayor en el número de bacilos viables que en la frecuencia de la respiración. Este fenómeno es considerado en relación con los fenómenos fotoinactivante y fotorreactivante ya descritos para la *E. coli*. También se considera en relación con el efecto observado de la luz sobre la reducción de la virulencia.

Se demostró que la albúmina ejercía un efecto protector acentuado sobre las suspensiones de BCG expuestas a una dosis letal de rayos procedentes de la lámpara solar.

RESUME

Les effets de l'irradiation aux U.V. sur la viabilité, la virulence et l'activité biochimique du Mycobacterium tuberculosis

L'irradiation aux U.V. du bacille tuberculeux a déterminé un certain nombre de phénomènes extraordinaires en ce qui concerne la viabilité, la virulence et l'activité biochimique de cet organisme.

Une corrélation étroite a été obtenue entre le degré de virulence des souches de *M. tuberculosis* (H37Rv, BCG et H37Ra) et leur sensibilité à l'action létale de l'irradiation aux ultra-violets des lampes à vapeur de mercure, de même qu'aux rayons ultra-violets de longueur d'onde encore plus courtes. Moins les souches étaient virulentes, plus était grande leur sensibilité à l'irradiation.

Une même corrélation a été également obtenue pour les cultures de cette même souche (H37Rv) dont les degrés de virulence étaient physiologiquement différents. Les cultures dont le degré de virulence était plus faible, soit parce qu'elles étaient plus vieilles soit parce qu'elles s'étaient développées en présence de paraformylacétanilide thiosemicarbazone, étaient plus sensible à l'action létale de la lumière.

L'irradiation produisait une réduction prononcée dans la virulence de la souche H37Rv à l'égard des souris. La réduction de la virulence paraissait proportionnelle à la dose de l'irradiation et représentait vraisemblablement l'altération photo-chimique directe de la masse de bacilles inoculée à la souris.

Une dose d'irradiation donnée déterminait une réduction plus marquée du nombre d'organismes viables que du taux d'oxydation. Ce résultat est considéré par rapport aux phénomènes de photo-inactivation et de photo-réactivation qui ont été décrits pour *E. coli*. Il est également considéré par rapport aux observations sur l'action de la lumière sur la réduction de la virulence.

L'action protectrice exercée par l'albumine sur les suspensions de BCG exposées à une dose létale de rayons U.V. a été démontrée.

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Case Report

ISOLATION OF ALLESCHERIA BOYDII FROM SPUTUM[1]

JOSEPH CREITZ AND H. WILLIAM HARRIS

(Received for publication July 26, 1954)

Allescheria boydii was first isolated from a case of maduromycosis in 1921 by Boyd and Crutchfield (1). This fungus was investigated in detail by Shear (2), who described it as a new species. In 1944, Emmons (3) showed *A. boydii* to be the sexual stage of the more widely known fungus, *Monosporium apiospermum*. *A. boydii*, the perfect or sexual form, is indicated by the presence of perithecia containing ascospores in addition to the asexual conidia which are found in both stages. According to Benham and Georg (4), perithecia are more often produced when *Monosporium apiospermum* is cultured on a medium with a reduced amount of nitrogen, such as 1 per cent Difco peptone, or 0.2 per cent asparagine.

A. boydii has been reported as the cause of two cases of maduromycosis (5) and one case of meningitis (4). There are two reports of the isolation of this stage from the soil (5, 6). *Monosporium apiospermum*, the asexual form, has been reported as the cause of twenty-one cases of maduromycosis (5), one case of septicemia (7), and, possibly, one case of otitis (8). In the case reported here, *A. boydii* was cultured repeatedly from the sputum. This is believed to be the first recorded isolation of this fungus from sputum.

CASE REPORT

A 56-year-old Spanish-American male, was admitted to the Veterans' Tuberculosis Section, Fitzsimons Army Hospital, in May, 1953. The patient had noted progressive fatigability, anorexia, and weight loss since July, 1952. He had had a chronic cough for twenty years but during the year prior to admission it had become productive of purulent sputum which was occasionally blood-streaked. At no time was the sputum noted to have a fetid odor. Ten months prior to admission a chest roentgenogram was abnormal, but the patient refused hospitalization. He had received penicillin for five days, ten months prior to admission, and for seven days immediately prior to admission. Except for symptoms of chronic rheumatoid arthritis present since 1934, the past medical history was not contributory.

Physical examination revealed a cachectic, chronically ill male. There was soft tissue swelling, tenderness, and pain on motion of the interphalangeal joints of both hands and of the elbows. Clubbing of the fingers and toes was present. The thoracic cage was increased in anteroposterior diameter. The breath sounds were bronchial in quality in both the apical and subapical regions anteriorly. Numerous fine, moist, inspiratory rales were audible throughout all areas of both lung fields. There were scattered rhonchi and wheezes on expiration throughout the lower lobes posteriorly. The remainder of the physical examination was not contributory.

The hematocrit, hemoglobin and erythrocyte determinations fell within the normal range. The leukocyte count was 14,050 per cu. mm., with 75 per cent polymorphonuclear leukocytes, 18 per cent lymphocytes, 5 per cent monocytes, and 2 per cent basophils. Cultures of the twenty-four-hour sputum concentrate for tubercle bacilli were negative on eleven examinations, and all Ziehl-Neelsen stains of the sputum for acid-fast bacilli were

[1] From the Laboratory Service and the Department of Medicine, Fitzsimons Army Hospital, Denver, Colorado.

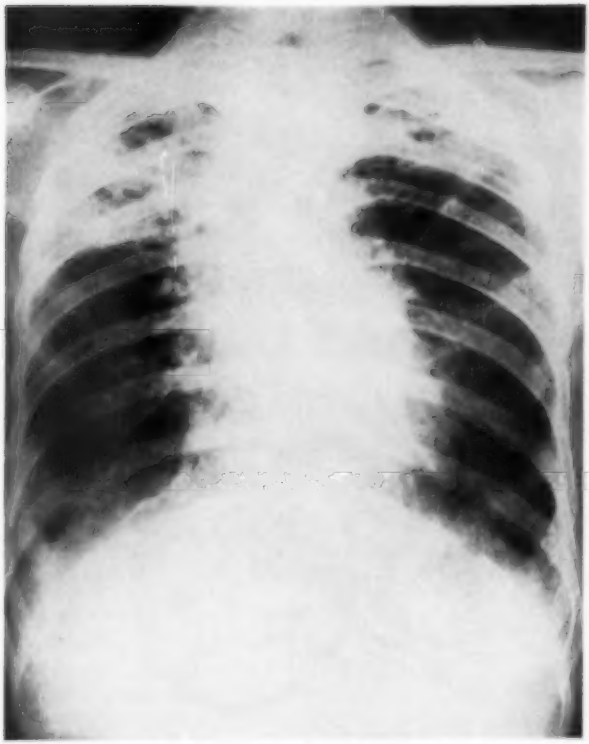

FIG. 1. Chest roentgenogram (January, 1954) showing contraction of both upper lobes with multiple areas of cavitation throughout superior half of both lung fields.

likewise negative. Cultures of the sputum for pyogens yielded alpha hemolytic streptococci, *Neisseria catarrhalis* and *Aerobacter aerogenes*. Intradermal skin tests with tuberculin (PPD) were negative in all strengths; intradermal tests with histoplasmin and coccidioidin antigen were also negative.

A chest roentgenogram (figure 1) showed contraction of both upper lobes with multiple areas of cavitation throughout the superior half of both lung fields. The largest cavity, which measured 8 cm. in diameter, was in the right upper lobe. There were scattered, soft, nodular, infiltrates throughout the upper halves of both lung fields. The lower lung fields appeared emphysematous. Bronchoscopy showed no evidence of endobronchial disease.

Initially the patient was thought to have advanced pulmonary tuberculosis, and he was given streptomycin, 2 gm. every third day, para-aminosalicylic acid (PAS), 12 gm. daily, and isoniazid, 300 mg. daily. This resulted in no improvement in symptoms, and serial chest roentgenograms showed no change. With the report of a negative tuberculin reaction and negative sputum cultures for tubercle bacilli, a diagnosis of chronic suppurative pneumonia with multiple lung abscesses was considered. The patient was then given penicillin by the parenteral and aerosol routes over a period of one month. This was followed by a long course of chlortetracycline (Aureomycin®) and, subsequently, oxytetracycline (Terramycin®). For one month he was given intermittent positive pressure breathing therapy. In spite of all therapeutic measures, the patient had no change in symptoms, and serial chest roentgeno-

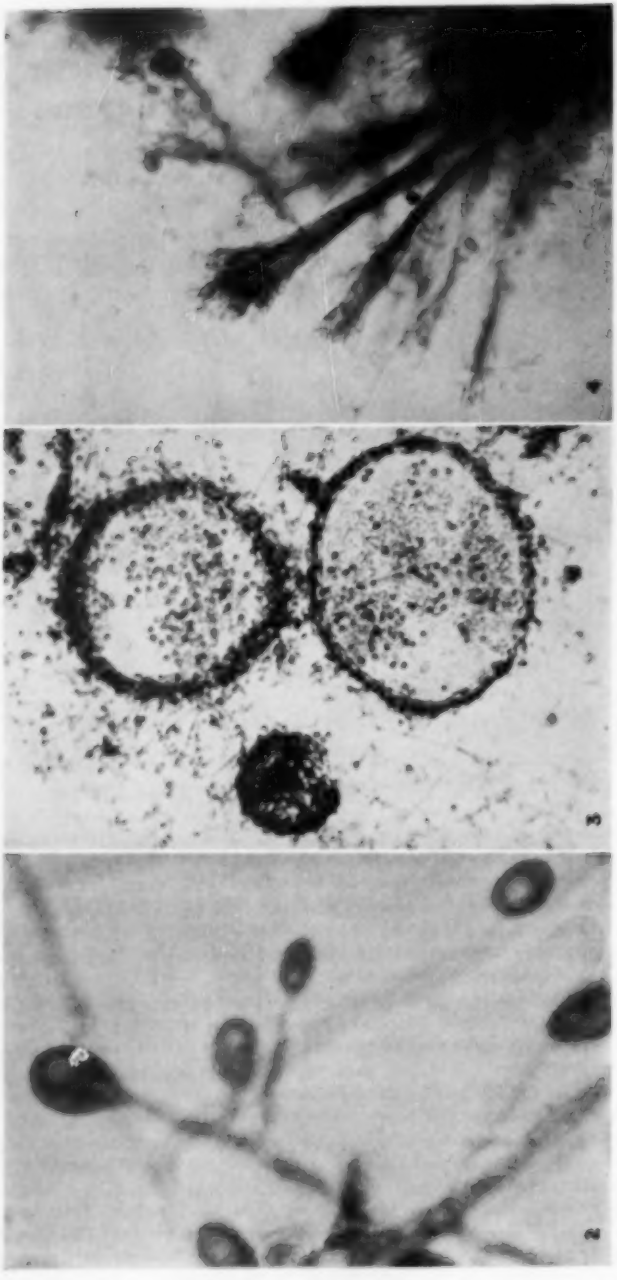

FIG. 2. Conidia showing refractile droplets. (×1000)
FIG. 3. Section of perithecia containing ascospores. (×200)
FIG. 4. Coremia and conidia. (×200)

grams showed only increased contraction of the upper lobes. The parenchymal infiltrates remained unchanged and the cavities persisted. The sputum continued to be profuse and purulent.

In January, 1954, each of six sputum specimens examined over a two-week period yielded a luxuriant growth of *A. boydii*. In February, 1954, the patient was transferred to another hospital.

Mycologic Findings

The fungus was initially isolated in the imperfect stage (figure 2) on brain-heart infusion agar with blood and antimicrobials added (9). When the organism was transferred to a medium containing 0.2 per cent asparagine, 4 per cent dextrose, and 2 per cent agar, cleistocarpous perithecia (figure 3) and coremia (figure 4) typical of *Allescheria boydii* were produced. In the young colonies the mycelia was white and woolly. The older colonies were brown and produced black pigment in the medium.

A single colony seventeen days old measured 58 mm. in diameter by 10 mm. in height. When cultured in brain heart infusion broth or Sabouraud's broth, the fungus produced a thick pellicle from which a weblike mass grew down to the bottom of the tube. The singly borne elliptical or clavate conidia varied somewhat in size, but averaged 4.5 μ by 7.5 μ. Refractile droplets in the conidia were observed to take Sudan III and IV stains, indicating their fatty nature. Perithecia measuring up to 230 μ in diameter were found in four-month-old cultures. The perithecia were filled with brown ascospores which were quite constant in size, averaging 3.5 μ by 6.5 μ. The elliptic [illegible] spores were much more constant in shape and symmetry than were the conidia.

COMMENT

Allescheria boydii infections are chronic in nature and progress very slowly, as was the case in this patient. As the fungus has been isolated from the soil, it seems probable that infection occurred by inhalation; however, it may have been present in the respiratory tract as a secondary invader.

At present there is inadequate evidence that the pulmonary disease was caused by this fungus. Proof of an etiologic role could be gained only by histopathologic examination of the lung tissue; however, the failure to demonstrate other pathogenic agents, and the lack of clinical improvement after prolonged therapy for chronic, suppurative lung disease, presents strong evidence favoring a fungus etiology.

SUMMARY

A case of chronic pulmonary disease has been reported in which *Allescheria boydii* was isolated from the sputum. This is the first known instance of recovery of this fungus in association with lung disease.

SUMARIO

Aislamiento de la Allescheria Boydii del Esputo

Preséntase un caso de neumopatía crónica en el que se aisló del esputo la *Allescheria boydii*. Esta es, en lo que se sepa, la primera vez que se haya descubierto este hongo en asociación con enfermedad pulmonar.

RESUME

Isolement de Allescheria Boydii dans le crachat

Il est rapporté un cas de maladie pulmonaire chronique dans laquelle *Allescheria boydii* a été isolée dans le crachat. Ceci représente le premier cas connu de récupération de ce fongus dans une affection pulmonaire.

Acknowledgement

The writers' sincerest thanks are extended to Dr. Norman F. Conant, Duke University, who concurred in the identification of the organism; to Mr. William Hommel of the Photography Section, Laboratory Service, Fitzsimons Army Hospital for the photographs; and to M/Sgt Ralph Hough for the preparation of the slide in figure 3.

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RECURRENCE OF COCCIDIOIDAL CAVITY FOLLOWING RESECTIONAL SURGERY[1]

LEROY HYDE

(Received for publication August 31, 1953)

Krapin and Lovelock (1) reported a patient who had a lobectomy because of bleeding from a coccidioidal cavity. Their patient was seen one year following surgery when recurrent coccidioidal cavities were noted in the remaining lobe of the same lung. The patient who is reported here had a segmental resection because of the mistaken diagnosis of "lung cyst." (This is the only pulmonary coccidioidal cavity to be excised among 17 such cases in the present writer's experience.) Follow-up examination ten months after the resection revealed a new cavity in the remaining portion of the same lung.

CASE REPORT

The patient, R. E. C., was a 22-year-old white male with a history of gradual weight loss of approximately 25 pounds, frequent colds, intermittent malaise, cough, and the expectoration of small amounts of brownish and mucoid sputum one year prior to admission. He denied chest pain and hemoptysis. A routine chest film was taken in April, 1948. The patient was told that he had a "congenital cyst." He was advised to have a follow-up chest film and this was obtained in September, 1948 (figure 1), at which time he was told that the "cyst" had enlarged and hospitalization was advised. As this patient lived in Southern California adjacent to numerous endemic areas for coccidioidomycosis, it was impossible to state exactly at what dates he had entered these areas.

The patient was 6 feet tall and weighed 140 pounds. The entire physical examination was within normal limits, and revealed an adult white male who did not appear acutely or chronically ill. Examination of the lungs was normal. The systolic blood pressure was 122 mm. and the diastolic pressure was 78 mm. of mercury.

The patient was afebrile during his presurgical course. Bronchoscopy revealed "minimal hyperemia and edema about the right upper lobe orifice with very minimal secretions."

The admission laboratory work yielded normal values. The urine revealed no abnormalities, and specific gravity was 1.027. The hemoglobin was 15.2 gm. per 100 ml. of blood; the erythrocyte count was 4.96 million cells per cu. mm.; and the leukocyte count was 9,600 per cu. mm., with 63 per cent neutrophils, 32 per cent lymphocytes, 3 per cent monocytes, and 2 per cent eosinophils. A serologic test for syphilis was negative.

The preoperative diagnosis was "infected lung cyst, left upper lobe," and on October 6, 1948, a segmental resection of the postero-apical segment of the left upper lobe was done. Pathologic examination of the resected segment revealed a cavity measuring 4 by 3 by 3 cm., surrounded by atelectatic lung tissue. The cavity communicated with a small bronchus which was completely obstructed, however, at its entrance to the cavity. A small amount of thin, gray, opaque, purulent material was noted within the cavity, which was lined by fibrinopurulent membrane. Microscopic sections were taken from the wall of the cavity and from the surrounding lung tissue. These revealed numerous areas of atelectasis and marked interstitial fibrosis and epithelization of the alveolar spaces. There were small foci of lymphocytes, and the pulmonary capillaries were moderately congested.

Foci of large round cells, plasma cells, and occasional polymorphonuclear leukocytes were seen in the peribronchial areas. One section showed two small tubercles, adjacent to a small bronchus. These changes were interpreted as compatible with a lung abscess.

[1] From the Pulmonary Disease Service, Veterans Administration Hospital, Long Beach, California.

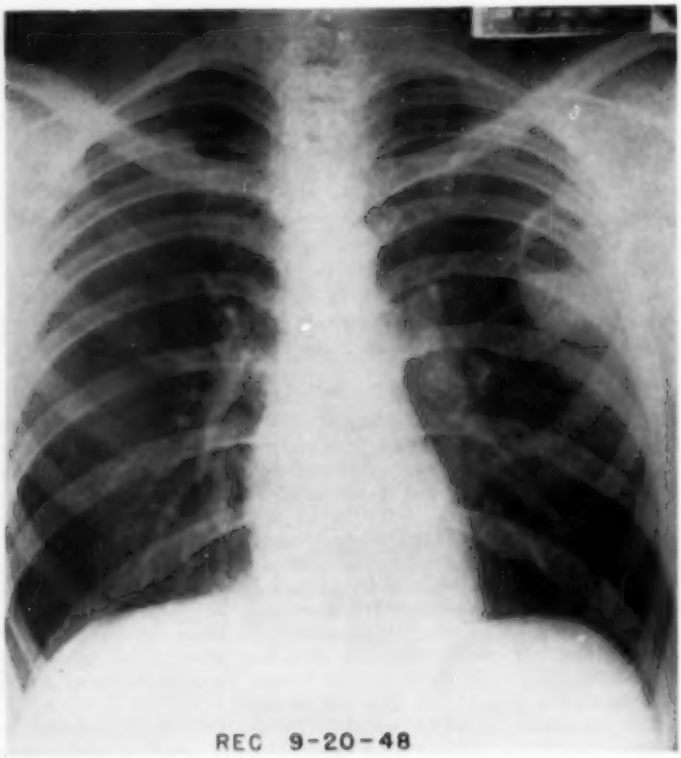


FIG. 1. Preoperative chest roentgenogram of September 20, 1948, reveals large left upper lobe cavity with fluid level, proved to be coccidioidal.

Three sputum concentrates and cultures prior to surgery were negative for tubercle bacilli. At the time of surgery, smears and cultures were procured from the cavity wall. A Gram stain revealed many pus cells but no organisms, and cultures for pyogens were negative. Cavity wall smears and cultures for tubercle bacilli were also negative. However, culture of the cavity wall revealed a heavy growth of *Coccidioides immitis*, and guinea pig inoculation with pus from the cavity also yielded *C. immitis*.

Two coccidioidin skin tests were done following surgery, one on November 9, 1948, using coccidioidin 1:100 dilution and the other on December 6, 1948, using 1:10 dilution; each test was negative in twenty-four hours. Several coccidioidal serologic studies were done through the kindness of Dr. Charles E. Smith. The first blood specimen was obtained on November 19, 1948, and the patient's blood agglutinated the sheep cells in the coccidioidal complement fixation test. Dr. Smith's interpretation was: "Suspect that this is a coccidioidal infection but would suggest another specimen."

Another blood specimen was obtained on December 9, 1948, and the complement fixation tests were 4-plus through the 1:8 dilution, and 3-plus in the 1:16 tube. Precipitin tests were negative. Serologic tests were repeated on January 11, 1949, at which time the complement fixation tests were positive (4-plus) up to 1:8 dilution of serum.

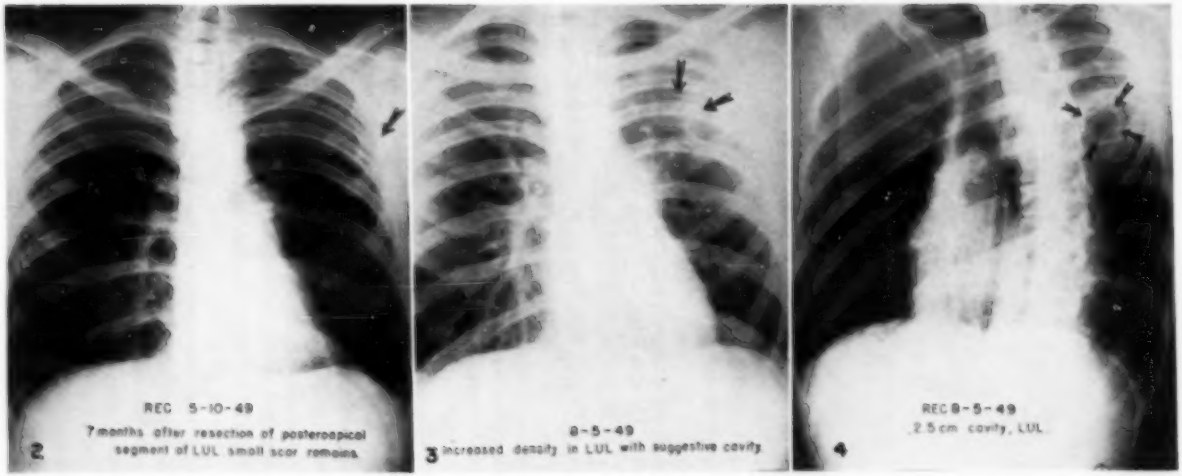
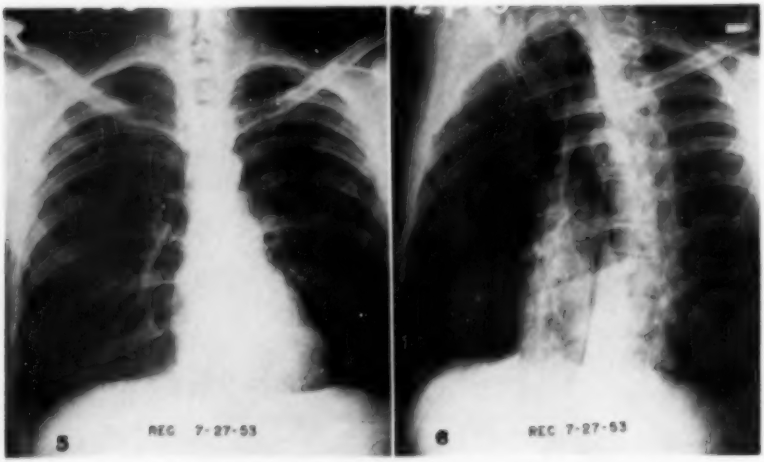


FIG. 2. Routine postoperative film, obtained seven months after resection of posteroapical segment of left upper lobe with coccidioidal cavity, reveals small fibrous scar.

FIG. 3. Chest roentgenogram of August 5, 1949, ten months after surgery, reveals new cavity.

FIG. 4. Left anterior oblique film of August 5, 1949, reveals cavity more clearly.



FIGS. 5 AND 6. Chest films of July 27, 1953, reveal disappearance of cavity. In left anterior oblique film, a small nodule may be seen at the site of the second cavity.

The patient had a relatively smooth postoperative course and was discharged from the hospital on December 6, 1948. He was followed at two-month intervals with serial chest films (figure 2). The film of August 5, 1949, revealed a large cavity in the remaining portion of the left lung (figures 3 and 4).

This patient was not seen again until called for a follow-up examination in 1953. He was asymptomatic and had been working since early 1949. The chest roentgenograms of July 27, 1953 (figures 5 and 6) and planigrams of the left upper lung field were normal. The cavity had evidently closed without any therapy. The complement fixation test for coccidioidal infection was positive (4-plus) through the 1:8 dilution of serum on July 27, 1953.

DISCUSSION

As Moore and co-authors (2) indicate, "There are many opinions as to the method of treatment of pulmonary cavitation in coccidioidomycosis. In some instances the patients have continued to work and the cavities have closed without any treatment." Other cases require lobectomy as did the two cases reported by these writers. The first case was asymptomatic and the comment is made, ". . . this case illustrates the innocent roentgenographic appearance and innocuous behavior of cavitation following primary quiescent coccidioidomycosis" (2). The second patient had respiratory symptoms including hemoptysis.

Greer and associates (3) reported nine lobectomies for persistent coccidioidal cavitation. Six of these patients had cavitation and debilitating symptoms. The three other patients had symptoms but they were not severe enough to keep them from their work. Seven patients were benefited from surgery, six working full time and one who was well twenty months after surgery but could not be followed further. Two of the original nine patients became worse than they were prior to surgery. One had developed pulmonary tuberculosis with cavitation and the other, two years later, had a return of symptoms and a new cavity in another lobe with sputum positive for *C. immitis*. This last case is similar to that of the patient being reported here. Greer and associates (3) recommended that "Lobectomy should be reserved for those patients who have distressing symptoms over a period of many months."

In the discussion of this paper (3), Dr. W. L. Rogers of San Francisco spoke of "the ordinary course of these residual cavities." Of a theoretical 100 cases, he stated that approximately 40 per cent would close spontaneously, 20 per cent would remain open but these patients would be relatively symptom-free, and the remaining 40 per cent would "require active therapy, either because of increasing size or because of recurrent, severe hemoptysis."

Cotton and Birsner (4) reported 30 patients with pulmonary coccidioidomycosis who had pulmonary surgery. Twenty-seven of these operations were for cavities. Associated conditions included pulmonary tuberculosis in 4 patients and carcinoma in one patient. One patient died postoperatively and another developed empyema. These authors recommended surgical treatment in "specific types of cavities: *a.* giant cavity, *b.* secondarily infected cavity, and *c.* blocked cavity." (4)

Other writers note that resectional surgery is required only rarely. Berke and associates have stated, "Non-intervention, therefore, should be the keynote of the therapy of these residual lung lesions. With only an occasional exception the condition is benign and the patient has few, if any, symptoms. Medicinal treatment is of no specific value. . . . We wish to emphasize that pneumothorax or other surgical intervention is not indicated, except in the infrequent case where there is significant hemorrhage from a cavity.". (5) The present writer is in full agreement with this point of view.

Indeed, in the present writer's experience (6), coccidioidal cavity is a benign entity comparable to a surgical scar or an anatomic defect. Seventeen patients with coccidioidal cavities have been followed by the writer for various periods up to eight years and they have had no difficulty from their pulmonary cavities. (The second coccidioidal cavity in the patient being reported was entirely asymptomatic and cleared without therapy.) The coccidioidal cavity is not dangerous either to the patient or to others. In non-coccidioidal areas, a spouse with a negative coccidioidin skin test does not develop cutaneous sensitivity while living with a patient who has a coccidioidal cavity. This indicates that the presence of the cavity is innocuous to others. Dissemination following the presence of a coccidioidal cavity is extremely rare. Willett and Weiss (7) reported one such case in a Negro patient. These episodes are believed to be most unusual. In a series of 274 cases of coccidioidal pulmonary cavities, Smith did not see a single case of dissemination (8). The more common situation by far is for the patient with the coccidioidal cavity to be asymptomatic. The cavity may vary in size on serial films and the fluid within the cavity may come and go. Neither of these changes appears to be significant. The chief indication for lobectomy because of a coccidioidal cavity would appear to be *recurrent severe* hemorrhage. Occasional blood-streaking requires no therapy other than reassurance of the patient. Dr. Charles E. Smith has stated (9) that "These patients (i.e., with coccidioidal pulmonary cavitation) have a high degree of immunity and very rarely disseminate. Most have no symptoms and rarely should anything be done to them. . . . Their danger of dissemination is negligible," (9) Owen (10) has said, "It must be re-emphasized that both nodules and cavities are benign manifestations of the primary phase of coccidioidomycosis, and that late dissemination from these foci is a most uncommon event."

SUMMARY

A case is reported of a patient who had a segmental resection for a cystic lung lesion which proved to be a coccidioidal cavity. Follow-up examination revealed a new cavity in the ipsilateral lung, which was asymptomatic and cleared without therapy.

Although some observers believe that excisional surgery is necessary in many cases of pulmonary cavitary coccidioidomycosis, others incline to the view that surgery is indicated only infrequently.

The present writer believes that a coccidioidal pulmonary cavity seldom requires any therapy other than reassurance of the patient. The cavity is not usually dangerous for the patient or for others. The greatest danger probably is iatrogenic disability.

SUMARIO

Recurrencia de Caverna Coccidioidal Consecutivamente a la Resección Quirúrgica

Preséntase el caso de un sujeto al cual se le ejecutó una resección segmental por una lesión pulmonar quística que resultó ser una caverna coccidioidal. El examen subsiguiente reveló una nueva caverna en el pulmón ipsilateral, que era asintomática y desapareció sin tratamiento.

Aunque algunos observadores creen que se necesita la excisión quirúrgica en muchos casos de coccidioidomicosis cavitaria pulmonar, otros se inclinan hacia la opinión de que la cirugía no está indicada más que raramente.

Al A. actual le parece que una caverna coccidioidal en el pulmón rara vez requiere tratamiento alguno, aparte de la confianza que hay que infundir al enfermo. La caverna no suele ser peligrosa ni para el paciente ni para los demás. El mayor peligro es probablemente la incapacidad yatrógena.

RESUME

Récurrence d'une caverne coccidioïdienne après résection

L'auteur rapporte le cas d'un malade ayant subi une résection segmentaire pour lésion systique du poumon qui s'est avérée une caverne coccidioïdomycosique. Des examens ultérieurs ont révélé la présence d'une nouvelle caverne dans le poumon du même coté; la lésion était asymptomatique et a guéri sans traitement.

Bien que certains auteurs soient d'avis que la chirurgie de résection soit nécessaire dans de nombreux cas de coccidioïdomycose cavitaire, d'autres sont enclins à penser que la chirurgie n'est qu'exceptionnellement indiquée.

L'auteur pense que la coccidioïdomycose cavitaire du poumon réclame rarement d'autre thérapie que celle qui consiste à rassurer le malade. La caverne ne présente habituellement aucun danger pour le malade ou pour son entourage. Le plus grand danger est probablement le trouble iatrogène.

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PULMONARY RESECTION IN SILICOTUBERCULOSIS[1]

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The over-all picture of the treatment of silicotuberculosis is a dismal one, and the experience with it at the Western North Carolina Sanatorium has been similar to that reported in the medical literature. Since 1947, 32 patients have been admitted to this hospital with a presumptive diagnosis of silicotuberculosis, half of whom were conclusively diagnosed as tuberculous by bacteriologic methods. Of this group of 16 patients, 10 have died; the lesions in 2 have been classified as "arrested," and, in an additional 2, the lesions have been considered to be inactive because of the prolonged disappearance of *M. tuberculosis* from the sputum. One patient is alive but is still discharging tubercle bacilli in the sputum. The case of the sixteenth patient forms the basis for the present report.

The coexistence of tuberculosis and silicosis has always presented a difficult problem for treatment. In general, such treatment has been limited to symptomatic relief. Moreover, the patient with silicotuberculosis could rarely look forward to a life expectancy of more than four years, even with the best in sanatorium care (1). Many of the time-tested procedures which are of great value in tuberculosis alone are of little or no value when the disease is combined with silicosis. Collapse therapy becomes a problem because of the resistant barrier to collapse which is produced by the marked fibrosis in the silicotic areas plus the loss of respiratory function produced by the destructive action of the combined pathologic processes on lung parenchyma (2). Because of the already reduced pulmonary reserve, excisional therapy is usually neither recommended nor feasible. It is not without reason, therefore, that Woodruff and his associates have stated that surgery is of limited value in the treatment of this condition, for any procedure which sacrifices lung tissue must necessarily increase the disability (3).

Bearing these hazards in mind, there are, nevertheless, some occasions when, in a patient with Grade I silicosis complicated by tuberculosis, surgical intervention has turned out to be of some value. Woodruff and Kelly have reviewed several reports on the removal of large, unilateral conglomerate lesions in patients with silicotuberculosis in whom the surgery was performed because the lesions could not be otherwise differentiated from neoplasms. It was their opinion that such surgery is not contraindicated if the mass is unilateral and the respiratory reserve adequate (3). Maier and Hurst have reported a single case in which an extrapleural pneumothorax resulted in improvement of a cavitary lesion (2). Theodos and Gordon have reported three cases of minimal silicosis with tuberculous cavities in which thoracoplasty was followed by closure of the cavities and reversal of infectiousness (1). In France, Bonniot has reported the successful segmental resection of unilateral cavities in seven cases of silicotuberculosis with no marked decrease in respiratory reserve during a short-term follow-up (4).

Because of the paucity of literature on the subject of pulmonary resection in silicotuberculosis, it was considered appropriate to report the successful resection of a cavitary lesion in a proved case of silicotuberculosis along with pre- and postoperative respiratory function studies.

[1] From the Western North Carolina Sanatorium, Black Mountain, North Carolina.

CASE REPORT

A 39-year-old white male was admitted to the hospital on August 27, 1951, complaining of increasing dyspnea, fatigue, and weight loss which commenced approximately one year prior to admission. Four months prior to admission, he had developed a cough with which he soon began to bring up large amounts of purulent, but odorless, sputum. His past history was not particularly eventful except for the fact that he had worked as a drill helper in a feldspar mine for fifteen years. He had also incurred an injury to his left elbow many years before which left him with a deformity of the left forearm.

On examination, he was found to be a poorly nourished, well-developed white male, not acutely ill or in obvious distress. His temperature was 98.6° F. His pulse rate was 76 per minute; his respirations, 20 per minute; and his blood pressure was 130 systolic, 98 diastolic in mm. of mercury. Except for slightly diminished breath sounds throughout the chest and the deformity of his left forearm as a result of the old injury, there were no abnormalities on physical examination.

A roentgenogram of the chest, obtained on admission, showed:

> General accentuation of the trunks throughout both lung fields, with some nodulation in their outline—probably due to silicosis, Grade I. . . . On the left, at the level of the first interspace, there is an area of density which is apparently due to tuberculous infiltration, within which there is a small area of cavitation.

Sputum examination on admission was positive for tubercle bacilli on microscopy.

Three weeks after admission, the patient was started on a regimen of streptomycin-PAS and continued on this medication for seventeen months.

Eighteen months after admission (February 26, 1953), this treatment was discontinued in favor of isoniazid-PAS because the patient's sputum had been negative for tubercle bacilli since October 7, 1952. Three months later (May, 1953) his sputum again became positive for tubercle bacilli while under treatment with isoniazid-PAS, and a roentgenogram of the chest showed the small cavity to be persistent and unchanged in the right upper lobe.

On November 10, 1953, the apical and posterior segments of the right upper lobe were resected. In general, the operation was performed with much less difficulty than had been anticipated. Nevertheless, the hilar dissection was made somewhat tedious by numerous fine adhesions which showed a marked tendency toward oozing and by the presence of multiple nodular implants about the hilum and mediastinal pleura which are characteristic of silicosis.

The pathologist's report on the resected specimen is:

> There is a focus of caseous necrosis surrounded by fibrosis with peripheral lymphocytic infiltration. In addition, there are nodular foci of fibrosis in which the fibroblasts are arrayed in whorls; and there are associated macrophages containing pigment granules.
>
> *Diagnosis:* Chronic granulomatous inflammation of the lung compatible with silicotuberculosis.

TABLE 1

RESPIRATORY FUNCTION STUDIES BEFORE AND AFTER SEGMENTAL RESECTION IN A CASE OF SILICOTUBERCULOSIS

	November 9, 1953	January 2, 1954	March 12, 1954
Minute volume	20.3 liters	29.0 liters	
Vital capacity	4.3 liters (in 5 seconds)	3.7 liters (in 5 seconds)	3.9 liters (in 5 seconds)
Predicted vital capacity	4.7 liters	4.6 liters	4.6 liters
Maximum breathing capacity	107.3 liters per minute	110.2 liters per minute	

Studies of respiratory function which were made immediately before surgery and two months after the operation are reported in table 1. It is believed that they demonstrate a minimal diminution of function by the operation.

The patient's course since surgery has been entirely uneventful. His sputum has remained negative for tubercle bacilli. During his hospital stay he showed a weight gain of 23 pounds.

SUMMARY

A case of silicotuberculosis in a young white male with unilateral tuberculous cavitation and Grade I silicosis was treated by segmental resection because of the reappearance of tubercle bacilli in the sputum and a persistent cavity after adequate medical therapy. With the removal of the cavity, tubercle bacilli disappeared from the sputum and the patient had an uneventful postoperative course. Studies of respiratory function before and after the resection revealed only a negligible diminution of respiratory function.

SUMARIO

La Resección Pulmonar en la Silico-Tuberculosis

Un caso de silico-tuberculosis en un joven de raza blanca con cavernas tuberculosas unilaterales y silicosis de Grado I fué tratado con la resección segmental, debido a la reaparición de bacilos tuberculosos en el esputo y a la persistencia de una caverna después de recibir terapéutica adecuada. Retirada la caverna, los bacilos tuberculosos desaparecieron del esputo y el caso tuvo una evolución postoperatoria sin complicaciones. Los estudios de la función respiratoria antes y después de la resección no revelaron más que disminución menospreciable de la misma.

RESUME

Résection pulmonaire dans la silico-tuberculose

Un cas de silico-tuberculose chez un jeune homme de race blanche présentant une lésion tuberculeuse cavitaire unilatérale et une silicose de "Degré 1" a été traité par la résection segmentaire en raison de la réapparition de crachats bacillifères et de la persistance de la caverne, en dépit d'une thérapie médicale adéquate. La caverne réséquée, les bacilles ont disparu des crachats et aucun incident post-opératoire ne s'est manifesté chez le malade. Les études de la fonction respiratoire avant et après la résection n'ont révélé qu'une diminution négligeable de la fonction respiratoire.

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Editorial

Can We Accelerate Tuberculosis Research?

In spite of the great advances made in the therapy of tuberculosis during the last few years, one has the feeling that tuberculosis research is not sharing the acceleration of pace so obvious in the general scientific world. Work is going on; some important discoveries are being made; a substantial number who have a genuine interest in tuberculosis research are working hard; but the intensity of effort, the diversity of approach, and the widespread interest of scientists of all disciplines necessary for rapid progress are wanting.

Yet, important leads for further investigation are not lacking. The opportunities for further rapid development and discovery are more numerous than ever before. When the possibilities are considered, one cannot escape the conviction that these leads should be followed with all the speed that is possible if we accept our full responsibility for the prevention of illness and death from tuberculosis.

It is unlikely that any two persons would agree on an order of priority, but a listing of some important fields which seem worthy of immediate attention will serve to emphasize the need for action now.

It was reported recently that a combination of pyrazinamide and isoniazid is capable of reducing populations of tubercle bacilli in infected mice to below the limits of detection by present-day cultural methods.[1] If this work is confirmed, it will be the first time any drug or any combination of drugs has been available which could consistently effect such a drastic reduction (or, possibly, even the complete elimination) of tubercle bacilli from the body of a susceptible animal. This observation has led naturally to clinical trials, and the therapeutic results have been highly encouraging; but this drug combination occasionally produces serious liver damage.

Those concerned with tuberculosis research should plan and stimulate a program to speed the investigations of the many questions raised by such an important discovery. Does it work in other animal species? What causes the liver damage? Can it be avoided? What is the optimum drug dosage? What are the effects on immunity and tuberculin sensitivity? How do the drugs work? The faster these and other questions can be answered, the sooner the benefits of improved therapy can be realized or the sooner we will know the reasons for failure. The possibility of such a radical improvement in drug effectiveness should justify a much larger expenditure of effort and money than is likely without stimulation.

We have assumed for years that there is a direct relationship of some kind between nutrition and tuberculosis; but we are still in ignorance concerning the specific nutrients which protect us from tuberculous disease or assist us in

[1] McDermott, W., Ormond, L., Muschenheim, C., Deuschle, K., McCune, R., Tompsett, R.: Am. Rev. Tubere., 1954, *69*, 319.

recovering from it. Here, again, we have some leads. A few studies have seemed to indicate that the vitamins *A* and *C* are highly important and may even have a specific action. It takes very little imagination to foresee the tremendous possibilities if we could obtain even a gross verification.

It is well known that laboratory procedures used to eliminate contamination from specimens kill as high as 90 per cent of tubercle bacilli which may be present and that culture media are far from perfect. Recently a method for decontamination with no lethal effect on the tubercle bacillus has been reported,[2] and a basic simple reproducible culture medium has been devised.[3] An immediate intensified testing program would seem justified to see that these improvements, if they turn out to be such, are put into general use without delay. The development of such things should not be left to chance.

It is now evident that strains of true tubercle bacilli differ in many particulars. Why is one strain virulent and another nonvirulent? What happens in the phenomenon of dissociation? What is the relationship of drug resistance to virulence? What are the genetic principles involved? Does bacteriophage play a part in virulence as has been observed with other bacteria? These and other questions have hardly been touched and probably have many practical implications.

Some have advocated the use of the tuberculin test in case-finding programs, particularly where infection rates are low. Without more factual data than we now have, who can say how effective or how expensive these procedures will be? Tuberculin testing programs have been carried on for years but we have few controlled studies from which practical conclusions can be drawn. There are many other epidemiological problems for which answers are equally as obscure.

Innumerable studies have been done in attempts to elucidate the mechanisms of immunity or resistance to tuberculosis. Many attempts have been made to develop a more effective vaccine. As disappointing as the results have been, the fact remains that, following inoculation of experimental animals with a variety of vaccines, an increase in immunity can be demonstrated. Further efforts are surely justified in the hope that more practical results eventually will be obtained. This problem should have the attention of more scientists in related fields, many of whom probably could be induced to join the tuberculosis research team.

This leads naturally to a consideration of newer tools which are not yet being used extensively in our field. Technical advances in the use of electron, proton, X-ray, phase, and reflecting microscopes, microsectioning, radioactive isotopes, tissue culture, single-cell culture, enzyme chemistry, and new statistical procedures to mention a few—should be actively exploited.

These few examples of the need for an accelerated tuberculosis research program bring us to some practical considerations. Research is expensive, and the investigators familiar with tuberculosis are few.

Tuberculosis research is carried on by universities, government agencies,

[2] Hirsch, J. G.: Am. Rev. Tubere., 1954, *70*, 312.

[3] Hirsch, J. G.: Am. Rev. Tubere., 1954, *70*, 955, 977, 989.

hospitals and others, with some of this research financed by the National Tuberculosis Association. However, the financial resources of the NTA are limited. Last year (1953) the gross return from the Christmas Seal Sale was approximately $24,000,000. One per cent of this, or $240,000, is required to be set aside for research, and this includes social research. An additional amount is added by the National Tuberculosis Association from its 5 per cent to bring this to $340,000 and the state and local associations will spend about $200,000 more. These sums represent the total financing of research by tuberculosis associations at present. There seems to be no possibility of escape from this financial straitjacket except through a larger seal sale or through curtailing other basic and effective activities.

On the optimistic side, there is a continuing interest in tuberculosis research and perhaps there are still some untapped resources if a plan of action can be set in motion. Informal conversations have indicated that many tuberculosis associations would be willing to contribute toward specific projects which show some promise of results of practical importance to the control of tuberculosis. It could also happen that the local support of carefully explained and well-publicized research would lead to an increased interest in the tuberculosis problem and a sizable increase in the sale of Christmas Seals. The key seems to be the word "specific." Contributions could be obtained if people knew exactly what their money was to be used for and why a particular problem needs investigating.

Because of its major purpose, its experience, and its tradition of pioneering, the National Tuberculosis Association and its medical section, the American Trudeau Society, might well make another major contribution by expanding the research program it has carried out so successfully in the past by the addition of an increasing number of projects of immediate practical application in therapy and prevention.

One of the first steps would be a delineation of the fields of research which have some chance of producing results in a reasonable length of time and which would appeal to the directors of tuberculosis associations and others who have funds at their disposal. The next step would be a search for qualified investigators who could be persuaded to undertake certain specific projects if they were assured of financial support. The third step would be to see that all who might be interested are given opportunities to support these specific research projects.

There has been much emphasis on the necessity for basic reasearch and, in a sense, this is a proposal to stimulate and extend those areas of tuberculosis research which have promise of some immediate practical benefit. It has been pointed out that some of the more brilliant discoveries were not the result of planned experiments, but it can also be pointed out that the additional researches to bring such discoveries to fruition were the result of long and arduous organized investigations. It has also been well demonstrated that additional effort will speed the tempo of research in a specific direction.

There is little point in arguing whether applied or basic research is to be supported. All basic research is done with the idea that eventually the knowledge

obtained will be useful. Any one who attempts applied research soon finds himself deep in basic research, and usually discoveries are made which have applications in fields other than that primarily being investigated. It still seems logical that progress in research will be faster and better if additional thought and effort are put into the solving of specific problems. Furthermore, the NTA has a mandate to use its money for the control of tuberculosis. This would seem to mean that research expenditures should be directed as much as possible to this end until tuberculosis is no longer of importance.

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Notes

EXPERIENCE WITH THE USE OF QUARTZ DUST IN AN ATTEMPT TO TEST THE VIABILITY OF TUBERCLE BACILLI IN RESECTED CLOSED TUBERCULOUS LESIONS FOLLOWING LONG-TERM ANTIMICROBIAL THERAPY

In an attempt to test the viability of tubercle bacilli demonstrated by microscopy in the lesions of treated patients, free crystalline silica was used *in vitro* and *in vivo* according to the method proposed by Vorwald, Dworski, and Pratt.[1] The lesions studied were closed tuberculous pulmonary lesions from 12 specimens resected from 11 patients who had received courses of antimicrobial therapy up to thirteen months in length. The free crystalline silica, having particles of 3 microns and less in size, used in this study was supplied by the Saranac Laboratory through the kindness of Dr. Vorwald.

Ten patients in this study who had received no previous chemotherapy were treated with the following antimicrobial agents in preparation for resection in the regimens indicated: isoniazid alone, 3; streptomycin-PAS, 3; streptomycin-PAS-isoniazid, 3; isoniazid-streptomycin, 1. One other patient received isoniazid-streptomycin-PAS with an interruption of one month between two courses of eleven and two months, respectively.

The duration of the courses prior to resection of the 12 specimens was as follows: less than four months, 1; four months, 2; four to eight months, 5; eight to ten months, 3; thirteen months, 1.

All specimens contained "closed" lesions which varied in diameter from 1 to 5 cm. and contained caseous material varying in consistency from liquid to cheeselike. The lesions in 9 specimens were described as filled cavities; the other 3 specimens were described as containing multiple small nodules. No specimen contained an open cavity.

Only specimens which contained material positive for acid-fast bacilli on microscopy were used in this study. Seven specimens were reported as showing acid-fast bacilli in tissue sections as well as on smears.

The technique used by Vorwald and co-workers for culture and guinea pig inoculation of the caseous or necrotic material was followed exactly except that the material was suspended in Proskauer and Beck medium (without serum or plasma enrichment) instead of saline solution.

The caseous material from all lesions in a single resected specimen was pooled for examination.

Material obtained from 4 specimens was positive for tubercle bacilli on culture: four of the positive cultures were obtained from suspensions of caseous material without the addition of quartz; and 2 positive cultures were obtained from material with quartz. The growth on Löwenstein-Jensen medium of the tubercle bacilli isolated from 2 of the 4 specimens was scant, and inoculation of these cultures into guinea pigs failed to produce disease. The growth of the other two strains of tubercle bacilli was luxuriant and both produced tuberculosis in guinea pigs.

Inoculations were made in tuberculin-negative guinea pigs which were selected at random from the general laboratory stock. Following inoculation, each animal was returned to a separate cage where it remained until it was sacrificed. The care and health of all animals in the colony were uneventful throughout the period of observation.

Two guinea pigs were used for each specimen. One received 1 ml. of macerated tissue in

[1] Vorwald, A. J., Dworski, M., and Pratt, P. C.: Am. Rev. Tuberc., 1954, *69*, 841.

Proskauer-Beck medium; the other received the tissue suspension plus 1 ml. of quartz suspension. Inoculation was made subcutaneously in the right flank area.

Animals were skin tested at one-, two-, and three-month intervals with 0.1 ml. of 3 per cent Old Tuberculin; all were negative at the test periods. Post-mortem examinations were performed three months from the date of inoculation on all animals. If the gross examination showed no abnormalities, a negative report was made. Local lesions developed at the inoculum site in 3 animals which received a silica-free inoculum and in 4 animals which received silica-enriched inoculums; but the lungs, liver, spleen, and retroperitoneal lymph nodes were normal in all but one.

Tissue was removed from the local lesions for smear, culture, and histologic study. In the 3 non-silica–treated animals, acid-fast bacilli, apparently of altered pathogenicity, were demonstrated in the smears, cultures, and sections. In the silica-treated group, one animal died of a typical, generalized tuberculosis. (However, the cultures of both the silica and non-silica suspensions of this specimen were positive for *M. tuberculosis*.) Another animal had a local lesion from which apparently modified acid-fast bacilli, similar to those found in the first group, were recovered; and 2 had local pyogenic infections.

In summary: The use of quartz dust failed to aid in the demonstration of viable tubercle bacilli by culture and animal inoculation in a small series of closed tuberculous lesions which were resected following preparation with antimicrobial therapy.

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THE HIDDEN CAUSES OF DISEASE. By ANTONIO BENIVIENI, translation by Charles Singer, bibliographical appreciation by Esmond R. Long. Pp. 217, Charles C Thomas, Springfield, Illinois, Price, $6.75.

We are now fortunate in having an excellent English translation of Benivieni's best known work, *De abditis nonnullis ac mirandis morborum et sanationum causis* (On some hidden and remarkable causes of disease and recovery). Benivieni, the Florentine physician, contemporary of Leonardo da Vinci, lived from 1443 to 1502 and recorded a series of brief case records, some with autopsy reports, in the fields of clinical medicine and surgery. The autopsy reports are some of the earliest recorded and earn for the author distinction as one of the founders of pathology. The book fills an important gap between the anatomical works of Mondino and the pathological anatomy of Morgagni.

Much pleasure is derived from diagnosing many of the conditions from the limited but vivid descriptions. The charm of each case report, however, lies in the insight that it gives into the practice of medicine and the problems encountered by a busy physician of the period.

Considerable attention has been given to the format. The book is easy to read, and for those who like to follow the original Latin version, this is included opposite the translation. The biographical sketch and photographs enhance the appreciation of the book. This work is recommended to all with an interest in the history of medicine.

RALPH L. ENGLE, JR.
New York, New York

THE CHEST. A HANDBOOK OF ROENTGEN DIAGNOSIS. Second Edition. By Leo G. Rigler. Pp. 380, The Year Book Publishers, Inc., Chicago, 1954, Price, $8.00.

All the rewarding features of the first edition of this compact volume are to be found in the second, and there have been some useful additions. The text is lucid and concise, the illustrations clear, the print easy to read, and the paper a pleasure to touch. The book shares with others of the same series the happy apposition of captions to illustrations, across the page at the same eye level. Clinical information is unobtrusive and to the point, serving as a pivot for the reader as he gropes through a differential diagnosis. The author describes how a set of films may be systematically examined, anatomy reconstructed, and artifacts recognized. A timely paragraph on radiation hazards and protection has been added, and the range and limitations of radiographic methods are discussed at some length, with the merciful omission of kymography. In his approach to each group of thoracic diseases, Dr. Rigler calls attention to the wide range of normal variation, perhaps the basic lesson of roentgenography.

Twenty-eight pages and twenty-five illustrations have been added to the second edition. To primary and metastatic tumors are given six more pages than previously; bronchography is considered in more detail, and a diagram has been included to show the divisions of the tracheobronchial tree. More space is given to tuberculoma, interstitial fibrosis, and erythema nodosum, and the "infiltrating carcinoma" of figure 234 of the first edition now emerges, after seasoned deliberation, as a histologically proved "multicentric, alveolar-cell carcinoma." The section on Tuberculosis, one of the pithiest in print, has been further tightened and improved. Through this section, more emphasis is placed on the importance of serial films in judging the activity of a lesion.

Two line drawings would have been welcome: one to show the distribution of the pulmonary segments in postero-anterior and lateral projection; the other to show how the patient should be positioned if these segments are to be filled during bronchography.

Dr. Rigler takes a most cautious view of angiography and speaks rather in passing of vascular disease. In such a handbook as this, he is to be congratulated for avoiding the maze of congenital heart disease, but could he not have devoted a page or two to cardiovascular disease now amenable to surgery, in which angiography can provide crucial information? What of coarctation of the aorta, cyanotic heart disease, arteriovenous fistula, and blockage of the vena cava?

These are trifling suggestions when measured against all the book offers in its new edition. For this is more than a guide book. It is a source book, pleasant to consult, in which the authority may find refreshment and the beginner a treasure of information.

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BOOKS RECEIVED

BIOCHEMISTRY AND HUMAN METABOLISM. Second Edition. By Burnham S. Walker, William C. Boyd, and Isaac Asimov, with a foreword by John T. Edsall. Pp. 904, The Williams & Wilkins Company, Baltimore, Maryland, 1954, Price, $10.00.

BROMPTON HOSPITAL REPORTS. Volume XXII, 1953. Pp. 165, Brompton Hospital, London.

BIOCHEMICAL DETERMINANTS OF MICROBIAL DISEASES. By René J. Dubos. Pp. 152, Harvard University Press, Cambridge, Mass., 1954, Price, $3.50.

MANUAL OF CLINICAL MYCOLOGY. Second Edition. By Norman F. Conant, David Tillerson Smith, Roger Denio Baker, Jasper Lamar Callaway, and Donald Stover Martin. Pp. 456, W. B. Saunders Company, Philadelphia and London, 1954, Price, $6.50.

BIOLOGY. Second Edition. By Claude A. Villee. Pp. 670, with illustrations, W. B. Saunders Company, Philadelphia and London, 1954, Price, $6.50.

AN OUTLINE OF PRESENT DAY THORACIC SURGERY. By Robert I. Carlson. Pp. 158, Educational Publishers, Inc., St. Louis, 1954.

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DIRECTORY FOR EXCEPTIONAL CHILDREN: Schools—Facilities—Services. Pp. 96, Porter Sargent Publishers, Boston, Mass., 1954, Price, $1.00.

A PRACTICAL MANUAL OF DISEASES OF THE CHEST. Fourth Edition. By Maurice Davidson, with the assistance of John H. Friend. Pp. 647, with illustrations, Oxford University Press, London, New York, Toronto, 1954, Price, $19.25.

MASTER SURGEON. By Ferdinand Sauerbruch. Pp. 277, Thomas Y. Crowell Company, New York, 1954, Price, $3.50.

GERIATRIC NURSING. Second Edition, By Kathleen Newton. Pp. 424, C. V. Mosby Company, St. Louis, 1954, Price, $4.75.

MEREDITH'S HYGIENE. Fifth Edition. By Arthur F. Davis and Warren H. Southworth. Pp. 906, The Blakiston Company, Inc., New York, 1954, Price, $6.00.

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NOTICES

ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY

The Fiftieth Annual Meeting of the American Trudeau Society will be held in Milwaukee, Wisconsin, beginning Monday, May 23. The meeting is in conjunction with the joint Annual Meetings of the National Tuberculosis Association and the National Conference of Tuberculosis Workers.

All program sessions will be held in the Milwaukee Auditorium. There will be no headquarters hotel. The December issue of the NTA *Bulletin* contains a list of the cooperating hotels and motels. Also included in the *Bulletin* is the registration form which must be used to obtain reservations at the hotel of your choice.

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES urges you to send in your reservations early. Additional copies of the registration form can be obtained from the NTA, 1790 Broadway, New York 19, New York.

* * * * * *

There are many scientific sessions planned by the Medical Sessions Subcommittee for Monday, Tuesday, and Wednesday, May 22, 23, and 24. The program for Thursday, May 25, has been planned for the combined interest of medical as well as non-medical persons attending the meeting. The preliminary program, containing subjects and speakers, will be published in the March issue of the *Bulletin*.

POSTGRADUATE COURSE IN THE MEASUREMENT OF PULMONARY FUNCTION

A postgraduate course in the measurement of pulmonary function in health and disease will be held for physicians in the New England area at Boston City Hospital, March 21–25, 1955, under the sponsorship of the American Trudeau Society, in cooperation with the Massachusetts Tuberculosis and Health League, Harvard Medical School, Boston University School of Medicine, and Tufts College Medical School.

The course is being held for the purpose of presenting the fundamental concepts and methods of analysis of normal and abnormal physiology of the lungs in a manner which can be readily understood by physicians unfamiliar with this type of chest work. Applications and more detailed information may be obtained from Edward J. Welch, M.D., chairman, ATS Regional Committee on Postgraduate Courses, 1101 Beacon Street, Brookline 46, Mass.

ANTIBIOTIC MEDICINE

Antibiotic Medicine, a new companion journal to *Antibiotics and Chemotherapy*, is being published by the Washington Institute of Medicine. The editor-in-chief is Henry Welch, PhD., and the associate editor is Felix Marti-Ibanez. The editorial board includes more than thirty well-known international authorities. The new journal will be exclusively devoted to clinical studies and practice of antibiotic therapy. The first issue will appear in January, 1955.

PUBLICATION OF "HOW TO STUDY NURSING ACTIVITIES IN A PATIENT UNIT"

The Public Health Service of the U.S. Department of Health, Education, and Welfare announces publication of *How to Study Nursing Activities in a Patient Unit*, a manual to aid hospitals in making better use of personnel. The publication offers a method by which

hospitals of all sizes may determine how nursing personnel time is distributed between duties requiring nursing skills and those which could be performed by other hospital personnel. The purpose of the study is to give nurses more time to be with patients. Nursing personnel themselves have an opportunity to take part in the study and to analyze their own activities.

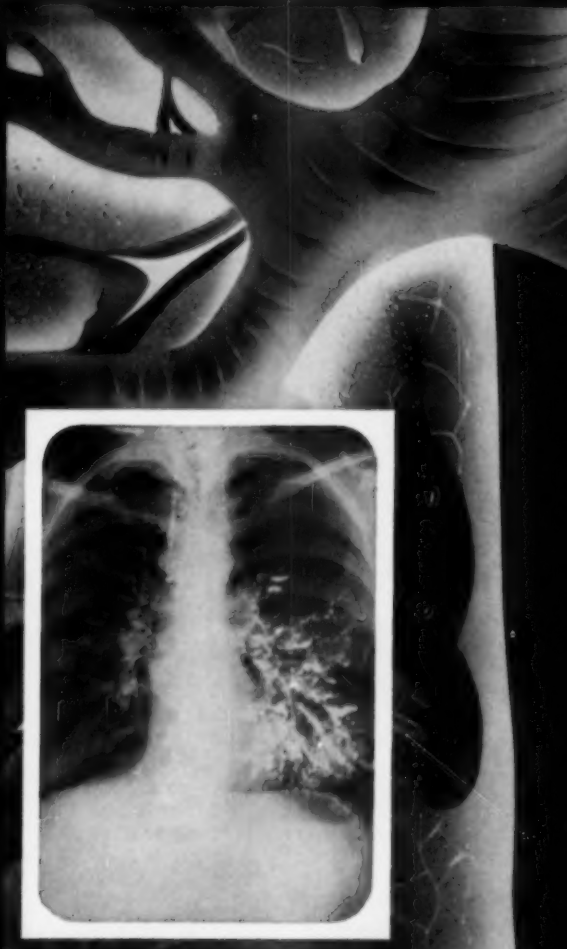
Prepared by the Division of Nursing Resources under the direction of Margaret G. Arnstein, R.N., Chief, the manual reflects the Public Health Service's concern with finding ways of making more nursing care available to the public through the conservation and more effective use of scarce nursing skills.

The manual may be purchased for 25 cents per copy from the Superintendent of Documents, Government Printing Office, Washington 25, D. C.

FOURTH COMMONWEALTH HEALTH AND TUBERCULOSIS CONFERENCE

The Fourth Commonwealth Health and Tuberculosis Conference will be held on June 21–25, 1955, at the Royal Festival Hall, London.

For further information, write to the Secretary-General, National Association for the Prevention of Tuberculosis, Tavistock House North, Tavistock Square, London, W. C. 1.



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Singer, J. J.: Differential Diagnosis of Chest Diseases, Philadelphia, Lea & Febiger, 1949, pp. 1953-4.

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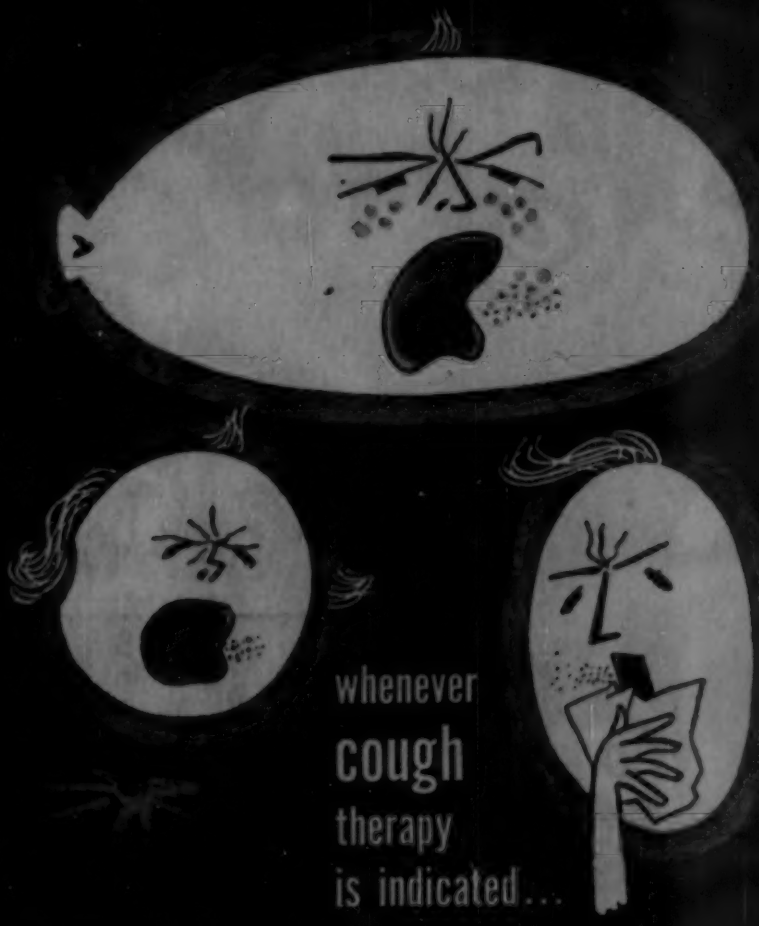

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